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JULY-DECEMBER, 1946

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ENDOCRINOLOGY

VOLUME 39 JULY, 1946 NUMBER 1

QUANTITATIVE EFFECTS OF HYPOPHYSECTOMY ON TESTIS AND PROSTATE OF DOGS

CHARLES HUGGINS AND PAUL S. RUSSELL[1]

From the Department of Surgery, The University of Chicago

CHICAGO, ILLINOIS

SOME of the chemical changes occurring after hypophysectomy in the prostate and testis of dogs are described in this paper. For several reasons it has become important to study the involution of normal tissues, particularly since the methods which induce regression in them can sometimes be applied to produce dysfunction in neoplastic cells; the endocrine glands, as implied by definition, exert remote control of certain other tissues.

Despite many and extensive studies of the results of extirpation of the pituitary, few quantitative data exist concerning its effect on the testis. The principal concern of previous investigators has been with changes in weight and cytology and with tissue metabolism.

Hypophysectomy causes a reduction in weight of the testis which varies in different species. In the cat (McPhail, 1935) the decrease is relatively small and the testis weighs about half its former proportion of body weight 47 days after hypophysectomy, while in the cock (Hill and Parkes, 1934; Allanson, Hill and McPhail, 1935) the testis decreases to about one-twentieth of normal. In the rat the testicular weight becomes about one-fourth (Crooke and Gilmour, 1938) and in the guinea pig and rabbit (Allanson, Hill and McPhail, 1935) about one-fifth of normal. Hypophysectomy in ferrets (Hill and Parkes, 1933) during "anoestrus" prevents the natural growth of the testis in the breeding season.

Cytologically the testis of the rat (Smith, 1930, 1939; Crooke and Gilmour, 1938) always shows several layers of spermatogonia with mitoses in the more peripheral cells. In the guinea pig (Allanson, Hill and McPhail, 1935) and ferret (Hill and Parkes, 1934) most tubules

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have a single layer of Sertoli nuclei and spermatogonia with occasional spermatocytes. In mature monkeys (Smith, 1938, 1939) at 30 days after removal of the pituitary only Sertoli-like cells and an occasional basally-placed spermatogonium are present in seminiferous tubules. The only data which exist concerning the testis of hypophysectomized dogs, the first species subjected to this operation, concern cytologic changes. When the operation has been done in pre-puberal dogs (Aschner, 1912) the testis remains retarded in development, the tubular lumen is filled with threads and secretion granules; the fat content of the Leydig cells is not strikingly altered.

In rat testis, oxygen utilization (Reiss, Druckrey, and Hochwald, 1933; Meier and Schuler, 1941) is decreased after hypophysectomy; also anaerobic glycolysis lessens and aerobic glycolysis increases.

MATERIALS AND METHODS

Physiologic. Hypophysectomy was done in 4 dogs by the method of Dandy and Reichert (1925) under intravenous anesthesia with sodium seconal, 30 mg. per kilogram. The dogs were large adult males so selected that the testes measured through the scrotum with calipers were identical in size. Removal of the gland was judged to be complete in these animals since no fragments of pituitary gland were found in the sella at necropsy and characteristic regressive changes were found in the adrenal glands, the average weight of a single adrenal at necropsy being 0.595 grams. At the time of hypophysectomy one testis was removed for chemical study and the opposite testis was recovered when the dogs were sacrificed 41 to 68 days after operation. Fat stains with Sudan dyes were made on frozen sections of the testes and hematoxylin-eosin sections were also prepared.

In 3 additional dogs removal of the pituitary was incomplete since minute fragments of tissue, identified histologically as pituitary, were found at necropsy.

In 3 normal dogs a wedge shaped section of the left lobe of the prostate was removed for phosphatase determinations and the testes were then removed; the right lobe of the prostate was assayed at necropsy about 2 months later.

Chemical. All of the testes were weighed and the content of water, fat and chloride determined as previously described (Huggins and Eichelberger, 1944) except that fat was extracted from the tissue by ethyl ether and petroleum ether for 8 hours in a Soxhlet apparatus. Acid and alkaline phosphatase content of the prostate was determined by the method of King and Armstrong (1934) in the hypophysectomized and castrate dogs as well as in 9 normal adults and 3 immature puppies. About 1 gram of prostate was minced and weighed in a tared weighing bottle and 10 cc. of water and 0.5 cc. of chloroform were added; the tissue was extracted in this way for 2 days at room temperature before phosphatase testing.

RESULTS

Complete hypophysectomy. All of the dogs gained in weight, from 0.6 to 3.4 kilograms, on average 2.1 kg. The pituitary glands weighed from 61 to 79.5 mg.

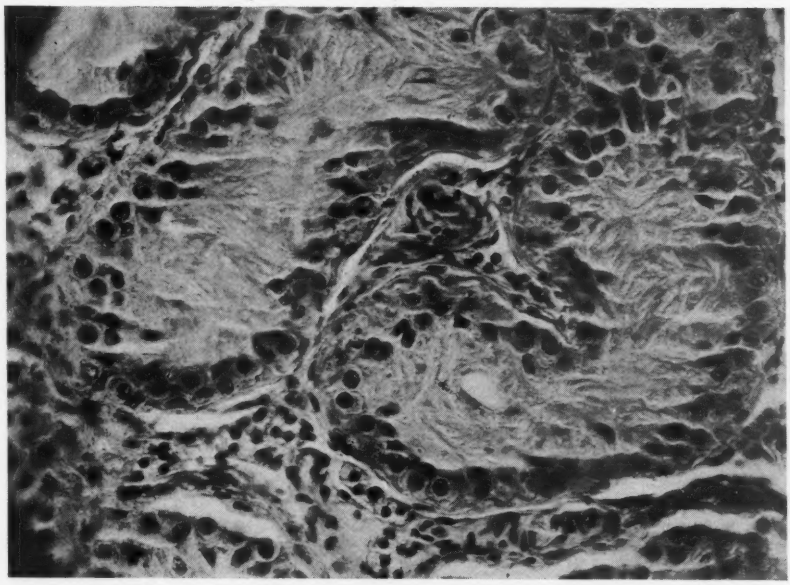

FIG. 1. Testis of completely hypophysectomized dog (8-49) 60 days after removal of pituitary. ×440.

All of the testes underwent a decrease of size and weight but the loss was not rapid. The testis at 41 days weighed 61 per cent of the opposite control but at 60–68 days the weight was from 35.1 to 37.9 per cent of normal.

The testicular tubules were filled with material appearing fibrous in paraffin sections. The tubules were lined with a single layer of sper-

TABLE 1. EFFECT OF HYPOPHYSECTOMY ON WATER, FAT AND CHLORIDE CONTENT OF TESTIS

Dog	Testis	State	Days	Pituitary, mg.	Testis				Prostate, gm.
					Weight, gm.	Water,* gm.	Fat,* gm.	Chloride,* mM.	
7-06	Right	Normal	0	61	13.5	850	15.4	64.2	
	Left	Hypophysectomy	41		8.25	856	25.8	81.7	3.52
8-49	Right	Normal	0	60	7.63	876	24.0	49.0	
	Left	Hypophysectomy	60		2.89	856	31.0	70.4	5.22
8-81	Right	Normal	0	68	14.0	868	18.3	53.7	
	Left	Hypophysectomy	68		4.92	861	24.4	74.8	3.0
9-39	Right	Normal	0	79.5	11.6	874	16.7	56.1	
	Left	Hypophysectomy	65		4.34	870	26.6	68.3	4.0

* Values expressed per kilo of tissue.

matogonia and Sertoli-like cells (Fig. 1); spermatocytes or more mature cells were not found. The interstitial cells appeared to be reduced slightly in size but staining with Sudan dyes showed that they contained abundant quantities of fat.

Chemical changes. After hypophysectomy (Table 1) the testes with one exception showed a decrease of water varying from 4 to 20 grams per kilogram. The total fat concentration increased from 0.6 to 1.04 grams per cent above the normal; this was only a relative increase

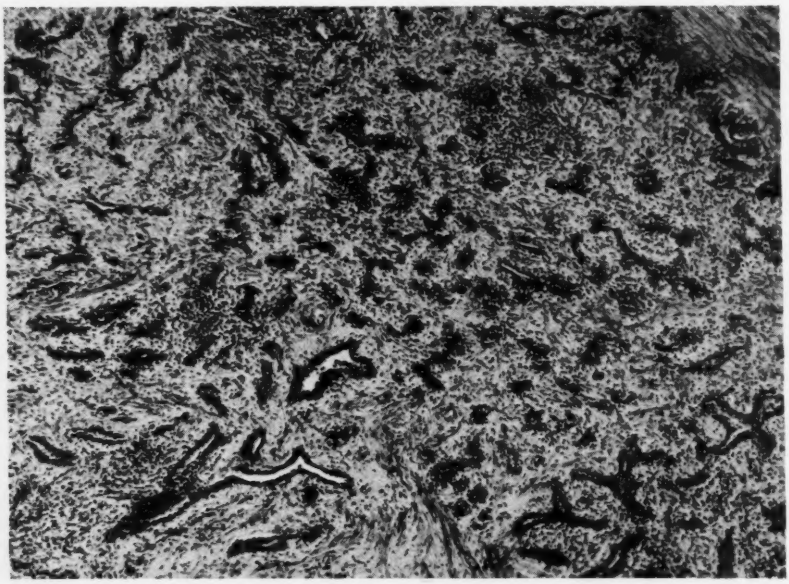

FIG. 2. Prostate of completely hypophysectomized dog (9-39) 65 days after removal of pituitary. ×135.

since the total fat content ranged from 46.8 to 59.6 per cent of the opposite testis. Chloride increased from 12.2 to 21.4 millimols per kilogram.

Changes in the prostate. The prostatic gland of hypophysectomized dogs was small, weighing 3 to 5.22 grams. Histologically the acini were practically obliterated (Fig. 2), the epithelial cells were basophilic and shrunken and seemed to possess no cytoplasm outside of the nucleus. The glands cytologically were slightly more atrophic than the prostate glands of 60 day castrates which contained occasional recognizable acini with small lumina and the content of enzymes was confirmatory of the histologic impression of profound atrophy.

The median content of acid phosphatase (Table 2) in 9 normal dogs was 26.5 King and Armstrong units per 1 gram; in prepuberal

puppies this value ranged from 0.3 to 1.73 units; acid phosphatase varied from 0.8 to 2.85 units in the hypophysectomized and from 5.2 to 11.5 units in castrate dogs, orchiectomy having been done 54 to 74 days previously.

The alkaline phosphatase of the prostate of castrate dogs was considerably increased above any other of the experimental groups (Table 2).

Incomplete hypophysectomy. In 3 dogs there was only a slight decrease of size and weight of the testis. About 2 months after hypophysectomy an examination of the sellar region revealed minute amounts of anterior pituitary tissue. In these dogs the prostate gland under-

TABLE 2. ACID AND ALKALINE PHOSPHATASE ACTIVITY OF DOG PROSTATIC GLAND
King and Armstrong units per 1 gram.

State	Number of dogs	Acid phosphatase			Alkaline phosphatase		
		Maximum	Minimum	Median	Maximum	Minimum	Median
Normal Adult	9	70	18.6	26.5	1.84	0.84	1.24
Prepuberal	4	1.73	0.35	1.07	1.74	0.43	0.89
Complete Hypophysectomy*	3	2.85	0.80	1.2	2.0	0.89	0.94
Castrate†	3	11.5	5.2	9.6	21.2	17.5	20.7

* 60 to 68 days postoperatively.
† 54 to 74 days postoperatively.

went atrophy while the production of spermatozoa in the testis was unimpaired. Typical findings are described in the following protocol:

Dog 7-72. On June 4, 1945, the right testis weighing 13.95 grams and the pituitary (75.3 mg.) were removed under seconal anesthesia. The recovery was uneventful. The dog was sacrificed 57 days after hypophysectomy, at which time the remaining testis weighed 12.78 grams and the prostate 6 grams. The prostate gland histologically revealed severe atrophic changes while the testis was completely normal with an abundant spermatogenesis with spermatozoa formation. In the sella, a small remnant of the anterior hypophysis weighing 8 mg. was found and confirmed cytologically.

DISCUSSION

Complete hypophysectomy produces changes in the testes of dogs comparable to those in the guinea pig in that a single row of spermatogonia survives in the tubules. The changes in the germinal epithelium are intermediate in degree between those reported in the rat and the monkey. In the hypophysectomized dog the testes remain in the scrotal position whereas in the other species (Smith, 1939) they become cryptorchid.

Hypophysectomy also produces the most profound atrophy that we have observed experimentally and the epithelium is reduced so that it resembles the prepuberal state both histologically and enzymi-

cally. Gutman and Gutman (1938, 1939) discovered that acid phosphatase in the prostate of monkeys is a chemical secondary sex characteristic whose activity is increased by androgen. In the testes of hypophysectomized dogs the fat content of interstitial cells is abundant; the concentration is increased although the total testicular lipid content is reduced. Thus there appears to be a dissociation between form (fat content) with function (androgen production) in the interstitial cells of the testis. Obviously since androgen is not being produced in physiologically significant amounts it is impossible to use the fat content of the Leydig cells as an index of androgenic formation.

The chemical changes in the testis after hypophysectomy, namely decreased water and increased chloride and fat are comparable to the changes which occur in other forms of testicular atrophy (Huggins and Eichelberger, 1944) as induced by estrogen or cryptorchism.

The retention of normal spermatogenesis in sub-totally hypophysectomized dogs indicates that a comparatively small amount of pituitary tissue will maintain the germinal function of the testis in this species, although androgen production is largely abolished. The findings are analogous to those of Allanson, Hill and McPhail (1935) in the guinea pig. Wells (1942) observed that spermatogenesis was present in 18.6 per cent of hypophysectomized squirrels.

CONCLUSIONS

Hypophysectomy in the dog is followed by a slight increase of body weight.

The testicular weight decreases to two-thirds of the normal value and is associated with decreased water and increased chloride and fat concentration. The total fat content is reduced. There is a dissociation between form and function in the Leydig cells, form and fat content being rather well retained while androgen production is greatly reduced or lost. Spermatogonia persist in the testis.

After hypophysectomy, the prostatic epithelium undergoes profound atrophy, being reduced to the prepuberal state. Prostatic acid phosphatase is decreased below the castrate level to values comparable with those found in juvenile animals. Alkaline phosphatase is decreased to prepuberal levels after hypophysectomy but is remarkably elevated after castration.

Subtotal hypophysectomy in dogs is associated with prostatic atrophy but with retention of normal spermatogenesis.

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A METHOD FOR LOCATING RADIOACTIVE ELEMENTS IN TISSUES BY COVERING HISTOLOGICAL SECTIONS WITH A PHOTOGRAPHIC EMULSION

L. F. BÉLANGER AND C. P. LEBLOND
From the Department of Anatomy, McGill University
MONTREAL, CANADA

IN CONTRAST to the considerable volume of biochemical investigation in tracing radio-elements in the body, little attention has been paid to their histological localization. A method, however, was developed by Lacassagne and Lattés (1924) for the demonstration of polonium in tissue sections. More recently, methods were devised to locate radioactive iodine in the thyroid gland (Hamilton, Soley and Eichorn, 1940; Leblond, 1943). In these various techniques, advantage was taken of the fact that photographic films or plates are sensitive to radioactive rays. If, therefore, histological sections of a tissue containing a radioactive element were juxtaposed to a photographic film or plate, an image appeared on the negative. A comparison of these images, so-called "autographs," to the histological slides themselves, demonstrated the localization of the radio-element.

Two factors, however, reduced the efficacy of these methods: 1) The contact between the histological section and the photographic emulsion was not intimate enough, even though various devices were used to press them together. This was at least one of the causes for the lack of sharpness in the negatives. 2) Furthermore, the matching of the autograph to the corresponding histological detail in the section was difficult, especially under high magnification.

This paper describes a method which reduces these difficulties. This was done by placing a photographic emulsion in direct contact with the tissue by spreading it over the histological sections themselves. The slides, which were now bearing both the section and the emulsion, were left in the dark for a variable length of time depending on 1) the type of ray emitted by the radio-element, 2) its half-life and 3) its concentration in the tissue. At the end of this period of exposure, the slides were developed and fixed according to the standard photographic procedure. A silver precipitate thus appeared wherever the emulsion was in contact with a zone of the section containing radio-elements. The slides were then stained, dehydrated and mounted in balsam. The gelatin emulsion did not interfere with dehydration and mounting, but greatly complicated staining, since the

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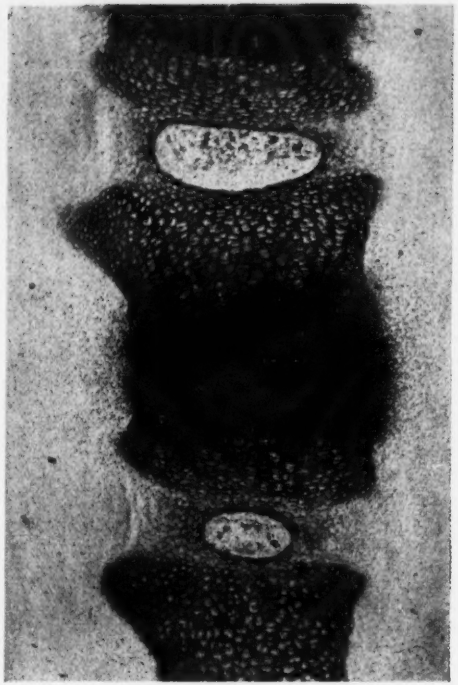


FIG. 1

FIG. 2

FIG. 1. Medium longitudinal section of tail vertebra (×50). In this preossification phase of development, the cartilaginous anlage of the vertebra shows an impregnation with radio-phosphorus in the central portion where the cartilage cells are degenerating. Stain: Methylene blue.

FIG. 2. Transverse section of the lower jaw, posterior region (×50). The membranous mandible is heavily impregnated with phosphorus. The molar tooth buds inside the bony arch show the phosphorus deposited in the dentine. Stain: Harris' Haematoxylin.

penetration of dyes and their differentiation were hindered and, in addition, the gelatin swelled in the staining solutions, thus lifting the sections away from the slides in a number of places. Satisfactory results, however, were obtained with methylene blue and Harris' hematoxylin for counterstaining radio-phosphorus autographs, and with Harris' hematoxylin for counterstaining radio-iodine autographs.

This new technique obviated the two main pitfalls of the previous methods, since an intimate contact between tissue and emulsion was obtained, and the matching of tissue and autograph was no longer a problem.

Satisfactory results were obtained with both radio-phosphorus and radio-iodine: 1) The radioactive phosphorus P^{32} was traced in the newborn mouse 45 minutes after subcutaneous injection of 0.05 mg. of sodium phosphate labeled with P^{32}. It appeared in periosteal and endochondral bone (fig. 3 and 4), degenerated and calcified cartilage (fig. 1) and dentin and enamel (fig. 2 and 5); 2) The radioactive iodine I^{131} was traced in the adult rat 45 minutes after its injection in 15 micrograms of sodium iodide. It appeared in the thyroid follicle (fig. 6).

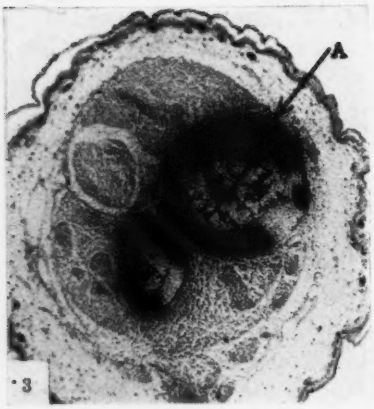
TECHNICAL DETAILS

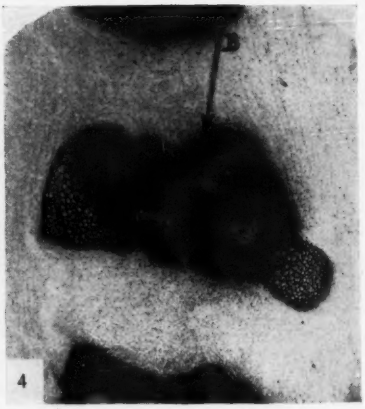
Commonly used *fixatives* did not interfere with the technique (neutral formalin, Bouin, etc.). Neutral fixatives had to be used when tracing radio-phosphates. Dehydration and embedding of the tissues were done according to routine histological technique, either through alcohol and xylol or through dioxane. The sections were cut at 10μ and attached to the slides with the usual egg albumen solution. After drying, the paraffin was removed in xylol, and the sections were carried through absolute alcohol to a 1% solution of celloidin in alcohol-ether. The excess was drained off the slides by standing them in an empty Coplin jar. They were left in 70% alcohol for about a minute to harden the celloidin and finally dried at room temperature.

The following operations dealing with the photographic phase of the techniques were carried out in the dark room at a distance of about 3 feet from a Wratten Safelight number one (Eastman). Lantern slide plates of the Medium contrast type (Eastman Kodak) were immersed in distilled water at room temperature. When the gelatin had swelled, the plates were removed from the water. The gelatin was scraped off with a glass knife and dropped into a small beaker. The gelatin was then melted by placing the beaker in a water bath at 35 to 40°C. Five drops of the melted emulsion were pipetted onto each prepared histological slide and spread evenly with a camel hair brush. This operation was carried out on a hot plate at approximately 39°C,[1] in order to avoid a too early solidification of the gelatin. In this man-

[1] Dr. Roger P. Loveland, Director, Photomicrography Department, Kodak Research Laboratory, called our attention to the fact that the temperature should be maintained below 40°C in order to keep down the fog.

Impregnated with phosp... the dentine. Stain: Harris' Haematoxylin.





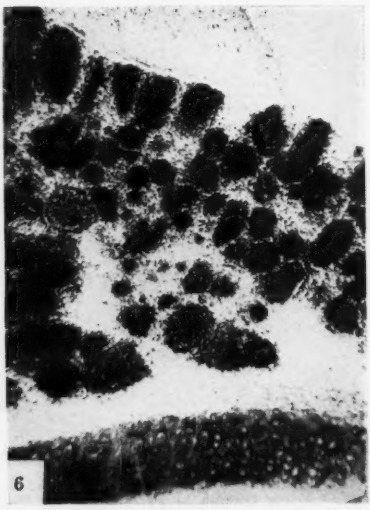


FIG. 3. Cross section through the lower limb (×50). The position of the radio-phosphorus is in the diaphysis of the tibia and the fibula. Arrow A points to heavy periosteal layer in the tibia.

FIG. 4. Para-median longitudinal section of thoracic vertebra (×50). Arrow B indicates the phosphorus deposit in the ossifying neural arch.

FIG. 5. Cross section of the lower jaw (×17). The deposition of radio-phosphorus clearly outlines the mandible. In the right portion of the bone an incisor tooth is developing and is also impregnated with the radioactive element.

FIG. 6. Section of the thyroid from an adult rat (×70), treated with radio-iodine. The tracheal cartilage is visible at the bottom of the figure. The thyroid follicles show a reaction due to radio-iodine.

ner, a thin, even coating of gelatin was obtained upon drying of the slide.

The emulsion-coated slides were then put away in a well closed container protecting them from light and dust, and in which they were kept horizontal. The length of exposure depending on the factors enumerated above had to be assessed for each individual case.

At the end of the exposure, the slides were removed from their container under Wratten Safelight number one and developed for 3 to 4 minutes in Kodak Developer D 72 at about 18 to 20°C. They were then washed rapidly in water and fixed for about 10 minutes in a 5% solution of thiosulfate at the same temperature. Finally they were washed in cold running water for about 30 minutes. The tissue sections were now transparent except in the zones of the slides containing the radio-element, where black silver deposits could be seen.

Staining procedures were carried out in Coplin jars cooled in running water. With methylene blue, the sections were left in a 1% alkaline solution for approximately 30 minutes and rinsed in running water until the stain was removed from the gelatin coating. With Harris' hematoxylin, the sections were usually lightly stained to avoid the necessity of differentiating them. The artefacts resulting from the swelling of the gelatin and the coming off of the sections disappeared when the slides were thoroughly dried after each operation. The procedure did not seem to affect the quality of the histological picture nor the autograph.

The slides were then carried through several changes of 95% absolute alcohol and xylol, and mounted in Canada balsam under a coverslip according to the routine histological procedure. Clearing in oil of origanum before mounting in balsam also gave excellent results.

SUMMARY

A new "autographic" method is described for the histological localization of radio-elements in animal tissues. It consists in covering the sections themselves with a photographic gelatin emulsion, which is developed after a sufficient exposure to the rays of the radio-element in the sections. Finally, the section is stained through the gelatin coating and mounted in the usual fashion.

ACKNOWLEDGMENT

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EFFECT OF THIOURACIL ON THE JUVENILE PLUMAGES OF BROWN LEGHORN FOWL[1]

MARY JUHN
From the Department of Poultry Husbandry, University of Maryland
COLLEGE PARK, MARYLAND

INTRODUCTION

THE INHIBITION of secretory activity with resulting hyperplasia of the thyroid which follows the administration of thiouracil was first reported in mammals (Astwood, Sullivan, Bissell and Tyslowitz, 1943; MacKenzie and MacKenzie, 1943). Later Mixner, Reineke and Turner (1944) showed significant weight increases of the thyroid in thiouracil-treated young chicks.

Thiouracil administration to the adult Brown Leghorn capon was found to effect changes in feather pigment and structure (Juhn, 1944a), which are wholly comparable to modifications following thyroidectomy (Greenwood and Blyth, 1927, 1929; Parkes and Selye, 1937; Domm, 1942).

In the plumage of the thiouracil-treated bird there develops a characteristic elongation of the feather with a reduction in barbulation, and a displacement of black by red pigments occurs, notably in certain regions of the body. Initially red pigments on the other hand remain relatively unaffected. These thiouracil-induced modifications may all be fully counteracted by simultaneous subcutaneous injections of thyroxine (Juhn, 1944b).

The description of the modified plumage of the thiouracil-treated capon may be extended at once to include the male, since the feathering of the Brown Leghorn cock and capon behaves similarly. In the hen, however, presence of the ovary is essential to the development of normal female feathering and the action of thiouracil in the plumage of this sex could accordingly become manifest through a diminished function of this gland or from immediate modifications in the feather papillae in the presence of reduced activity of the thyroid.

Although the two sexes of the Brown Leghorn show these distinct plumage determinations, males and females are alike in exhibiting a time order during their growth periods in which normal juvenile plumages succeed each other. This time order is unaffected by procedures which otherwise demonstrate the alternative plumage potentialities of the two sexes. In early ovariotomy, the developing pullet shows

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successive male stages of the comparable age (Pézard, Sand, and Caridroit, 1925). Following the administration of estrogens to chicks of both sexes from time of hatching, the male develops female feathering of the corresponding female age stage; the feathering of the female remains normal and is not accelerated toward maturity by this treatment (Juhn, Gustavson, and Gallagher, 1932). These records indicate the clear autonomy of the timing of these sequences from modifications of sex hormone levels and the observations suggest an examination of these orders in either sex under conditions of continuous thiouracil administration from an early age, since a participation of the thyroid in developmental regulatory processes is recognized. Experiments along such lines are described in the following.

PROCEDURE

The interest of the current observations centers predominantly in the induced plumage reactions under conditions of continuous presence of thiouracil from an early age. For the tests, twelve male and twelve female chicks were selected from a group of common ancestry. These were hatched April 24, 1944, and raised under identical conditions at the University Farm. The chicks were brought into the laboratory at seven weeks and three days of age, confined in batteries, and after a preliminary observation period of two weeks, one group of six males and one group of six females were placed upon a continuous thiouracil diet[2] by adding 5 gm. of the drug in 1000 cc. tap water to 1000 gm. of the dry feed, or 0.5 per cent[3] dry weight basis of feed intake. For the two control groups tap water was mixed with the feed in the same ratio.

The administration of thiouracil was commenced July 2, 1944, and surviving individuals continue under experimental conditions and under observation.

RESULTS

At the time of the preliminary observations, on June 29, 1944, the birds in all the groups were in full juvenile moult with very numerous feather germs in the breast and saddle. All males showed black breast feathers of juvenile shape and rusty-red juvenile saddle feathers; all females were in juvenile feathering.

At six weeks of thiouracil feeding, the treated males continued to show black breast feathers similar to the controls; the saddle feathers, however, were definitely retarded. The treated females showed no deviation from the controls at this same period.

At two months of thiouracil administration, both sexes had de-

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[2] The thiouracil used in these experiments was obtained through the kindness of Dr. Mark Welsh, Lederle Laboratories, Inc.

[3] The effectiveness of this proportion of thiouracil in the diet had previously been determined in the feathering of the adult Brown Leghorn capon (Juhn, 1944a). The daily dosage of thiouracil by weight in this case is approximately 0.4 gm. as such a fowl consumes 80–85 gm. of feed, dry weight, per day. Domm and Blivaiss (1944) later fed this same dosage of 0.4 gm. thiouracil as a dry powder enclosed in a gelatin capsule also to Brown Leghorn capons and found a similar response.

veloped bright orange-red feather tips in the contour feathers. The treated males also showed an extension in area of the normal rust-red segments of the secondaries in the wings and this male color appeared in the secondaries of the treated females in place of the female pattern.

During later periods of thiouracil-feeding, regional differences in the plumage reaction became progressively apparent; the stage described in the following is at eight months of treatment, when there survived six test males and five controls, five test females and five controls. There were more or less pronounced differences between members in each of the two experimental groups but these were rather in degree of response and not sufficient to warrant individual treatment.

The control males were in full adult plumage in which the breast is solid black and the dorsal tracts are orange-red with each feather marked with a black central stripe or black basal segment.

The control females also were in full adult plumage in which the breast is salmon, shading to buff stipple in the posterior regions. The dorsal tracts are buff stippled.

The thiouracil-treated males showed a remarkable diversity in the regional expression of the reaction to thiouracil. Certain feather tracts became completely altered and came, in fact, to resemble those of an entirely distinct region (compare figure 6 with figure 7);[4] some showed only partial deviation from the normal, while others persisted as an earlier juvenile stage. All the contour feathers of the body which were originally black became orange-red in the feather vane proper, the fluff remaining a soft gray. Simultaneously, the feathers increased in length and decreased in width and the rounded outline changed to a long narrow shape with a lacy margin replacing the solid edge because of the decreased barbulation. The modified red varied somewhat in shade between regions. the feathers of the hock being of a distinctly more brilliant tint. In the feathers of the dorsal tracts, the original red was little modified; however, the black sections of the vane tended to disappear; the fluff continued gray (figure 8). All these feathers and those of the posterior regions of the body especially showed an elongation and narrowing with loss of barbulation, often to an extreme degree.

The main tail feathers sometimes formed a faded brown segment in the shaft but were otherwise unchanged; this tract is most resistant to modifications, as has been found in other connections (Zawadowsky 1926). The wing feathers under thiouracil treatment showed the most interesting behavior. The group of shortest, black coverts both of the upper and the lower surface of the wing became a most brilliant orange, notably in the distal wing tips. The appearance of this vivid coloring was recorded in 1927 by Greenwood and Blyth in the young

[4] The color photographs for this paper were prepared by Mr. John Spurbeck to whom the author wishes to express her appreciation and indebtedness.

thyroidectomized Brown Leghorn. The primaries, the flight feathers attached to the hand, were in the adult stage, with only a slight extension of the normal light-brown edge with somewhat of a total narrowing and elongation. At the junction of the primaries and the secondaries, is the first of the flight feathers attached to the forearm, No. 11. Normally this feather differs from its neighbors in its smaller size; among the experimental males it invariably was represented as an earlier, juvenile stage. The adjoining feather, No. 12, also was juvenile but of a later stage. The next adjacent secondaries were adult with somewhat of a tendency toward elongation and increase in the area of the red-brown marginal segments. The more medial feathers among the secondaries, however, again were represented as juveniles and, furthermore, the stage shown became progressively earlier in feathers closer to the body to include the proximal-most of the tertiaries, the feathers attached to the upper arm. This condition as such was common to all the test males but the respective number of the feathers represented as adults and as juveniles differed among the experimental individuals.

As an exception to the general tendency toward the displacement of the black by red feather pigments, it is to be noted that sporadically certain groups among the normally parti-colored red-and-black feathers of the dorsal tracts laid down full black in the presence of thiouracil. Occasionally also black segments were laid down in otherwise red feathers comparable to effects produced by single subcutaneous injections of thyroxine.

The thiouracil treated females showed induced plumage modifications that were fully as complex as those described for the experimental cockerels. In the test pullets, feathers of the breast tract showed the effect of the drug in the resulting elongation, narrowing, and decreased barbulation but the pigment, while sometimes slightly deeper in tint, corresponded fairly closely to the original female salmon (compare figures 1 and 2). The persistence of this female salmon in the face of altered structure was also recorded for the thyroidectomized Brown Leghorn hen by Parkes and Selye (1937).

In the dorsal tracts, on the contrary, modified feathers corresponded in color to those of the treated male and also were similar in structural modifications although no comparable extremes in length were achieved (compare figures 5 and 8). In the thighs and hocks, the structural changes were typical: the color in the first region of the modified male reddish; in the latter, rather more of the female salmon. All the contour feathers of the thiouracil-treated females showed a tendency toward the appearance of transverse grayish or black bars, possibly indicating fluctuations in effective levels. The main tail feathers were female, sometimes showing some juvenile traits. In the smallest coverts along the edge of the wing there developed the same brilliant orange found in the test males. The primaries were somewhat

elongated and showed toward the very base of the vane some male red. In the secondaries, No. 11 had become long and very narrow with a female stippled tip; the adjacent feather No. 12 quite consistently resembled the thiouracil-modified male in color and shape. The neighboring secondaries were adult female but showed increasing male traits with increasing proximity to the body. The tertiaries exhibited a mixture of male and female juvenile traits. The coverts immediately over the secondaries were entirely female while those covering this row were modified male in appearance.

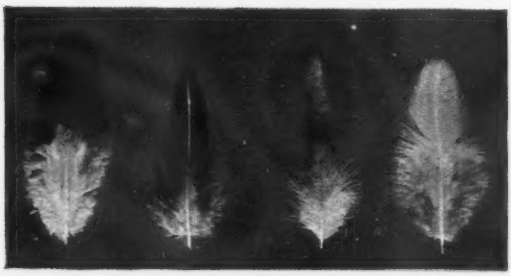
DISCUSSION

The description of the successive stages developed in the plumages of the thiouracil-treated birds shows that under conditions of this experiment, the response effected by the drug differed clearly in the separate plumage regions of both male and female. The continued development of juvenile characteristics in the feathering of treated individuals which were past the chronological age of maturity shows, first, that the juvenile sequences depend for their orderly expression on a normal function of the thyroid, and second, that the modified plumage continued to show some of the regional differences in reaction that are an important physiological attribute of the normal feathering. It is of considerable interest that these differentials persist under conditions of lowered metabolic level from a relatively early age.

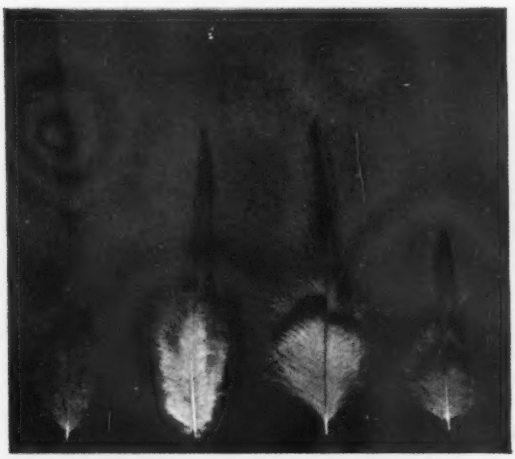
In the final stages, the male tended to assume the all-over appearance of a reddish, extremely silky bird but close inspection consistently showed the presence of black, notably among the flight and tail feathers where individual members resembled a normal adult pattern although having a somewhat attenuated form. The corresponding females also developed a silky red in the dorsal feathering, in general of a more subdued tint, the ventral regions retained much of the female coloring, and such feathers as progressed to the adult stage in wing and tail were of female pattern. The localization of the adult female pattern in the test hens suggests that ovarian secretory activity is not very greatly reduced[5] in this long-time thiouracil treatment at the dosage levels employed, since the respective plumage regions normally would be among the first to register falling female hormone levels by a male reversion. The drug, therefore, through its action on the thyroid appears to affect the feather follicles directly, probably through an altered morphology imposed upon the zone of growth of the feather germ, the collar.

The persistence, in either sex, of feather areas which fail to show a complete modification by thiouracil may indicate that it is impossible

[5] The plumage is subject to modification only during the growth periods of its components. The feathers described, whether showing characteristic thiouracil reactions in pigmentation and structure, or laid down as continued juvenile but of a later stage, all represent individual feathers that were moulted and replaced during thirouacil administration.



Colored films reading left to right.
Film A) FIG. 1. Normal female breast feather.
FIG. 2. Thiouracil-modified female breast feather.
FIG. 3. Normal female dorsal feather.
FIG. 4. Normal male breast feather.



Film B) FIG. 5. Thiouracil-modified female dorsal feather.
FIG. 6. Thiouracil-modified male breast feather.
FIG. 7. Normal male dorsal feather.
FIG. 8. Thiouracil-modified male dorsal feather.

to depress the activity of the fowl thyroid below certain levels as has been found by Astwood and Bissell (1944) in the rat. On the other hand, it is not fully clear from results stated in the literature for complete thyroidectomies in fowl whether these procedures lead to more incisive reactions: the descriptions are primarily concerned with the contour feathers, which agree with the thiouracil-modified birds. The published plates and the descriptions of bodily transformations also seem to correspond to the appearance of thiouracil-treated fowl.[6]

The plumage modifications which develop in the wake of thiouracil action in the male are more simple than in the female, and they afford some suggestions on the relation of the red to the black feather pigments in this sex. Red tends to replace black in diminished thyroid activity; the process is reversed when thyroxine is injected subcutaneously. Paschkis et al. (1944) have shown that melanin formation in a tyrosinase-tyrosine system is inhibited by thiouracil among other substances and they suggest that this may be through a direct anti-enzyme action. If these *in vitro* observations can be extended to Leghorn pigment behavior *in vivo*, decreased melanization here would follow interference with enzymatic oxidations. However, the Brown Leghorn fowl, among numerous breeds investigated by Bohren, Conrad and Warren (1943 pp. 506–7; 511), varies in that its red pigment is similar to the black in behavior toward acid solvents and in spectrophotometric analysis, so that this bird may prove a special case from which broader generalizations can not be drawn.

SUMMARY

Thiouracil in tap water was added in the proportion of 0.5 per cent feed, dry weight, and fed to male and female Brown Leghorn chicks commencing at seven weeks of age. At a period when controls were in full adult plumage, test males showed red feather pigments and reduced barbulation with an associated feather elongation in place of solid black contour feathers and a regional retention of juvenile stages in the wings. Certain feathers were of adult pattern but of attenuated shape.

Test females developed feathers similar to the modified male among the dorsal tracts, structural changes with but little altered pigmentation in the breast tracts, and showed in the wing feathers a regional distribution of female juvenile, modified male, and adult female pattern of an attenuated shape.

[6] As adults, treated birds of both sexes were very small and "blocky." They somewhat resembled the bantam variety in conformation and gave an impression of solid weight when handled. Males and females remained clearly recognizable and they had not come to approximate a neuter condition. Lay of eggs by test females, while irregular and scanty, was not fully interrupted. Test birds of both sexes developed anaemia: the erythrocyte number in the males was 1,830,000 per cu. mm.; in the females it was 1,950,000. Corresponding normal figures are approximately 3,500,000 and 2,500,000. The test fowl in these counts (6 males and 5 females) are too few for an evaluation of the apparent reversal of the normal dimorphism in erythrocyte number.

These results may be compared to those obtained in thyroidectomies.

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PITUITARY-GONAD INTERACTION IN IMMATURE FEMALE PARABIOTIC RATS[1]

ROLAND K. MEYER, CLYDE BIDDULPH, AND JOHN C. FINERTY

From the Department of Zoology, University of Wisconsin
MADISON, WISCONSIN

WHEN a normal immature female rat is united in parabiosis with an ovariectomized partner the ovaries of the intact rat undergo hypertrophy. This has been demonstrated by Kallas (1930), Hertz and Meyer (1937), Bunster and Meyer (1938), Biddulph, Meyer and Gumbreck (1940), and others. This ovarian stimulation has been attributed to the hypersecretion of pituitary gonadotrophic hormones which follows gonad removal. According to Zeckwer (1940), the typical castration changes in the pituitary gland of the castrated partner are unaltered by parabiosis, while the normal partner shows varying degrees of degranulation of both acidophil and basophil cells.

The parabiosis technique has been used as a method for determining the effect of injected gonadal hormones on the secretory activity of the pituitary gland, and also to ascertain the natural hormonal interactions of pituitary gland and gonads. It has been found that the injection of small amounts of estrogen or androgen into the castrated partner of any sexual conbination in parabiosis effectively inhibits the usual gonadal hypertrophy of the intact partner (Bunster and Meyer, 1933; Hertz and Meyer, 1937; Meyer and Hertz, 1937; Cutuly and Cutuly, 1938; and Biddulph, Meyer and Gumbreck, 1940).

The experiments to be described in this paper were designed to obtain data concerning the effect of estrogen on the gonadotrophic hormone content of the blood and pituitary gland of ovariectomized parabiotic rats, and to correlate them with the percentage of cell types in the anterior pituitary gland.

METHODS AND PROCEDURE

The experiments include observations made on a total of 104 pairs of female parabiotic rats, and 274 single female rats of the same age. All of the animals were obtained directly from Sprague-Dawley, Inc.

The operative procedure used in uniting the animals was that described by Bunster and Meyer (1933), except that metal skin clips were used in closing the skin wounds instead of silk sutures. All operations were performed under sterile conditions and under ether anaesthesia. Littermates weighing

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[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

70 grams or more were used, and were united at 31 days of age, the right partner being ovariectomized at the same time. In those pairs that were treated with estrogen, the ovariectomized partner was injected subcutaneously within 2 to 3 hours after the operation with 0.01 cc.of corn oil in which 0.055 ug. of estradiol was dissolved. The injections were made once daily for 10 days with autopsy on the morning of the 11th day of parabiosis. This dose of estradiol has been shown to be effective in inhibiting ovarian hypertrophy of the normal partner (Biddulph, Meyer and Gumbreck, 1940). Fifty-three pairs in which the left partner was intact and the right partner ovariectomized were used as uninjected control female pairs.

Studies were made of 60 ovariectomized single rats which had been treated with 0.055 ug. of estradiol per day from the 31st to the 41st day of life, 58 ovariectomized untreated (ovariectomized on the 31st day of life), and 56 normal single female rats. To determine whether or not the trauma associated with uniting the rats in parabiosis would affect the gonadotrophic activity of the pituitary gland, 50 ovariectomized and 50 normal female rats were subjected to a sham parabiosis operation in which incisions were made through the skin and body wall, followed by closure of the tissue without uniting the animals.

The animals of all groups were autopsied at 41 days of age, at which time the pituitary glands of each group were removed and dried on glass plates (Kupperman, Elder and Meyer, 1941). Aqueous suspensions were prepared from this dried pituitary powder and injected into 21 day old Sprague-Dawley female rats. Each recipient animals was injected once on the afternoon of the first day and twice daily for the next four days. Each animal received a total of 3 mgs. of dried powder. Autopsy was done on the morning of the sixth day.

The pituitary glands of 5 animals from each group were selected at random and fixed in Bouin's fluid, sectioned, and stained with a modified Mallory's trichrome stain for cytological study. The average percentage of each cell type was determined by differentially counting the cells in every tenth field of 3 horizontal sections at equidistant levels in each gland.

RESULTS AND DISCUSSION

The data obtained at the time of autopsy of the parabiotic and single rats are included in table 1. Although data are included on the weight of the adrenals, thymus, thyroid and pituitary, no particular attempt will be made to discuss the significance of these values. It is noteworthy that the weight of the thymus of the parabiotic rats was greatly decreased as compared to that of single rats. This is believed to be due in part to the effect of the extensive trauma associated with uniting the animals in parabiosis. It will also be noted that the weight of the thymus of the intact rats in parabiosis with an ovariectomized rat was considerably less than that of a rat in parabiosis with an ovariectomized parabiont receiving estrogen. This effect is thought to be the result of the action of large amounts of estrogen secreted by the ovaries as they were stimulated to increased activity by gonodatrophin from the pituitary gland of the untreated ovariectomized parabiont. The quantity of estradiol injected was sufficient to cause

some involution of the thymus as is shown by comparing the thymus weight of ovariectomized single rats given estradiol with that of rats not injected.

A study of the adrenal weights shows that parabiosis and/or ovariectomy caused a slight hypertrophy of the adrenals, which is of questionable significance. The effects of gonadectomy of the rat on the adrenals have been adequately described (Hatai, 1915; Anderson and Kennedy, 1933; Winter and Emery, 1936; Biddulph and Meyer, 1941; Parkes, 1945; and Smith, 1946), and show that in the male the adrenals hypertrophy, whereas in the female there is no effect or a slight decrease in weight. It would seem that the effect of trauma (Selye, 1936, a,b) is also a factor in the present study in accounting for the

TABLE 1. THE EFFECT OF OVARIECTOMY AND ESTROGEN TREATMENT ON THE AVERAGE WEIGHT OF VARIOUS ORGANS OF IMMATURE FEMALE RATS

Treatment	No. of rats	Body weight (gm.)	Ovarian weight (mg.)	Uterine weight (mg.)	Adrenal weight (mg.)	Thymus weight (mg.)	Thyroid weight (mg.)	Pituitary weight (mg.)
Untreated	56	114.3 ±0.9[1]	18.8 +0.6	74.7 ±4.4	25.6 ±0.4	400.1 ±8.4	9.1 ±0.4	4.5 ±0.2
Ovariectomized (10 days)	58	108.6 ±1.0	—	30.3 ±1.3	27.3 ±0.5	421.8 ±9.4	9.1 ±0.6	5.5 ±0.2
Ovariectomized plus .055 ug. estradiol per day for 10 days	60	100.3 ±0.7	—	115.1 ±1.6	25.0 ±0.3	347.6 ±6.1	8.8 ±0.5	5.5 ±0.2
Untreated Parabiotic rats:								
Left partner (intact)	53	89.0 ±1.1	131.3 ±6.0	—	26.1 ±0.4	170.6 ±6.2	8.2 ±0.6	5.1 ±0.4
Right partner (ovariectomized 10 days)	53	85.4 ±1.4	—	—	28.9 ±0.4	231.1 ±11.8	7.1 ±0.6	4.4 ±0.3
Estrogen-treated Parabiotic rats: (.055 ug. estradiol per day for 10 days)								
Left partner (intact)	51	84.0 ±1.3	16.5 ±0.5	—	27.4 ±0.1	234.6 ±12.0	6.7 ±0.5	3.9 ±0.2
Right partner (ovariectomized 10 days)	51	81.8 ±1.3	—	—	29.1 ±0.6	209.4 ±10.2	6.7 ±0.5	4.6 ±0.2

[1] Standard error.

slightly heavier adrenals of the operated animals. It should be pointed out that the injection of estrogen prevented the slight adrenal hypertrophy of the ovariectomized single group. Lauson, Heller and Sevringhaus (1937) observed no effect of estrogens on the size of the adrenals in ovariectomized rats, whereas Selye, Collip and Thomson (1935) obtained enlargement of the adrenals with estrogen only when corpora lutea were present.

The thyroid glands of single animals were somewhat heavier than those of parabiotic rats. The pituitary glands of the estrogen-treated ovariectomized or ovariectomized single rats were heavier than those of untreated single rats. Hypertrophy of the pituitary gland occurred in intact rats in parabiosis with uninjected ovariectomized parabionts, probably because of the large quantity of estrogen produced by the ovaries of the intact parabiont. Comparison of the uterine

weight of intact single rats with that of ovariectomized and ovariectomized estrogen-treated rats shows that 0.055 ug. of estradiol stimulated the uterus to greater growth than was found in the intact single rats. That a growth-promoting substance, estrogen, was secreted by the ovaries of the rats between 31 and 41 days of life is indicated by the smaller uterus in the ovariectomized single rats as compared to intact single rats.

The body weights of the single rats were greater than those of the parabiotic rats, which probably is an effect of the trauma associ-

TABLE 2. THE EFFECT OF OVARIECTOMY AND ESTROGEN TREATMENT ON CELL TYPES AND GONADOTROPHIC CONTENT OF THE PITUITARY GLAND OF SINGLE AND PARABIOTIC FEMALE RATS

Treatment	Average Ovarian wt. (mg.)	Pituitary No. of rats	Pituitary Assay Data Average Ovarian wt. (mg.)	Av. % Acid.	Cell Type Av. % Baso.	Analysis Av. % Chrom.
Untreated	18.8±0.6[1]	15	26.0±3.2	23.8±0.6	6.5±0.4	69.7±1.0
Ovariectomized (10 days)	—	14	39.0±6.1	23.7±1.7	19.7±0.4	56.6±1.4
Ovariectomized plus .055 ug. estradiol per day for 10 days	—	14	77.0±6.3	29.3±0.7	9.7±0.3	61.0±0.9
Untreated Parabiotic rats:						
Left partner (intact)	131.3±6.0	16	11.0±0.4	20.2±0.3	4.0±0.3	75.8±0.5
Right partner (ovariectomized 10 days)	—	12	28.0±5.0	23.6±0.7	11.6±0.5	64.8±0.7
Estrogen treated Parabiotic rats: (.055 ug. estradiol per day for 10 days)						
Left partner (intact)	16.5±0.5	10	25.0±4.6	23.9±0.8	7.7±0.2	68.4±0.8
Right partner (ovariectomized 10 days)	—	13	49.0±4.3	30.4±0.4	9.1±0.3	60.5±0.4

[1] Standard error.

ated with parabiosis and the abnormal condition under which parabiotic rats live. However, assay of the pituitary glands of the rats which were traumatized but not paired showed that such treatment did not change the gonadotrophic activity from that of normal single and ovariectomized single animals of the same age.

The data concerned with the percentage of cell types and gonadotrophic content of the pituitary gland of parabiotic and single rats, together with the ovarian weights of intact and parabiotic rats, are presented in table 2. These data are also represented graphically in figure 1.

The ovarian weight of intact rats in parabiosis with ovariectomized rats was 700 per cent greater than that of untreated single rats of the same age. The stimulated ovaries were composed of numerous follicles and many corpora lutea. This result is a well known one which has been reported in the literature many times, and is due to the increased amount of gonadotrophin which was secreted into the blood stream of the ovariectomized parabiont and transferred to the blood of the intact rat. It should be emphasized, however, that gonado-

trophin circulating in the blood of the ovariectomized parabiont is largely follicle stimulating in its effect (Greep, 1940; Biddulph and Meyer, in press). The luteinization of the ovaries of the intact parabiont was due to the additive effect of the gonadotrophin in the blood of the intact rat with that from the ovariectomized rat, or as it has

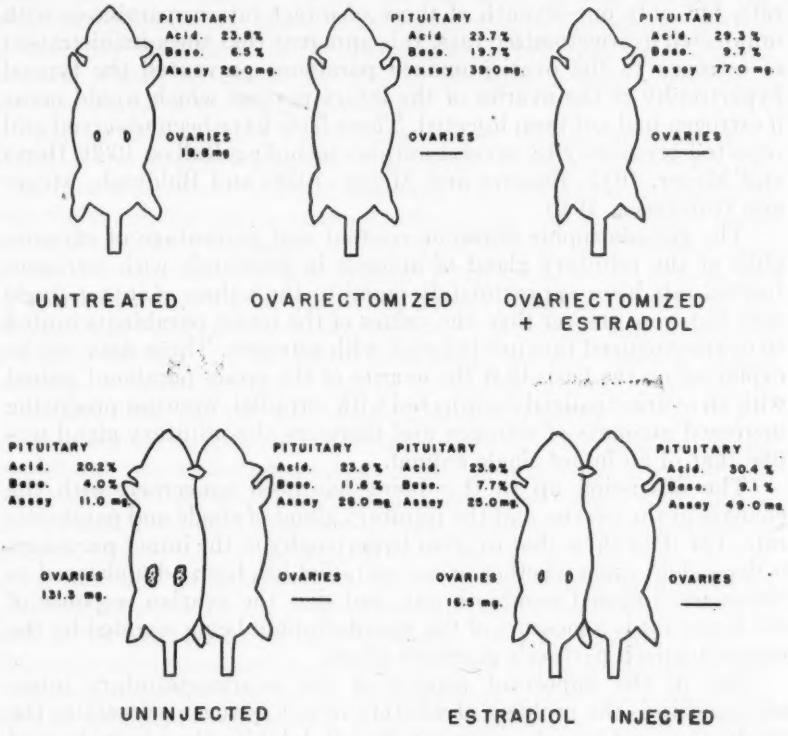

FIG. 1.

usually been explained, by the release of LH from the pituitary of the intact rat (Hohlweg, 1934; Fevold, Hisaw and Greep, 1936).

The gonadotrophic content of the pituitary of the intact parabiont united with an ovariectomized rat was less than that of the pituitary of an intact single rat, as is shown by the smaller weight of the ovaries of the assay rats. (Compare 11.0 mgs. with 26.0 mgs.).

The percentage of basophils and acidophils of the pituitary of the intact parabiont with stimulated ovaries was less than that of the pituitary gland of a single intact rat and less than that of any other rats used in this study. Thus, less gonadotrophin and a smaller percentage of chromophils was correlated with large stimulated ovaries

in the intact parabiont. These facts suggest that *large* amounts of estrogen and/or progesterone decrease the production of gonadotrophin by the chromophils.

The ovaries of intact parabionts united to ovariectomized females injected with 0.055 ug. of estradiol per day were unstimulated and weighed 16.5 mgm. which is approximately the same as those of single rats, but only one-seventh of those of intact rats in parabiosis with uninjected ovariectomized rats. It is apparent that the administration of estrogen to the ovariectomized parabiont prevented the typical hypertrophy of the ovaries of the intact partner which would occur if estrogen had not been injected. These facts have been observed and reported previously by several authors including Kallas, 1930; Hertz and Meyer, 1937; Bunster and Meyer, 1938; and Biddulph, Meyer and Gumbreck, 1940.

The gonadotrophic hormone content and percentage of chromophils of the pituitary gland of animals in parabiosis with estrogen-treated rats were approximately equal to the values of intact single rats, but were greater than the values of the intact parabionts united to ovariectomized rats not injected with estrogen. These data can be explained on the basis that the ovaries of the intact parabiont paired with an ovariectomized rat injected with estradiol, were not producing increased amounts of estrogen and therefore the pituitary gland was like that of an intact single animal.

The discussion up to this point has been concerned with the changes in the ovaries and the pituitary gland of single and parabiotic rats. The data show that ovarian hypertrophy in the intact parabiont is dependent upon whether or not estradiol has been administered to the ovariectomized parabiotic rat, and that the ovarian response of the intact rat is a measure of the gonadotrophin being secreted by the ovariectomized partner's pituitary gland.

One of the important aspects of the ovarian-pituitary interrelationship is the problem of whether or not estrogen stimulates the production and/or release or whether it inhibits the production of gonadotrophin. The data in this paper which bear particularly on this question are those concerned with the effect of estrogen on the chromophils and gonadotrophic activity of the pituitary glands of ovariectomized parabiotic rats injected with estradiol, and ovariectomized parabiotic rats not injected. The pertinent data are presented in table 2 and figure 1.

Before discussing this data it seems best to present an assumption which seems logical but which cannot be definitely proved at this time. It appears likely that in parabiotic rats there exists a series of concentration gradients of gonadotrophin between the cells of the pituitary gland of the ovariectomized parabiont (highest) and the ovaries of the intact partner (lowest). The first gradient is between the pituitary of the ovariectomized parabiont and the surrounding blood,

the second between the blood of the ovariectomized animal and the blood of the intact partner as the exchange of blood between the two is small, and the third between the blood and ovary of the intact partner. The last gradient may be due to the removal of the gonadotrophin from the blood by the ovary, as has been suggested by Heller, Heller and Sevringhaus (1942). On the basis of this concept the gonadotrophins in the blood of an ovariectomized parabiont are less in quantity than those in the blood of an ovariectomized single rat because of their dilution in the blood of two animals and their removal by the ovaries of the intact parabiont.

As the data in table 2 and figure 1 show, the gonadotrophic activity of the pituitary gland of an ovariectomized parabiont was less than that of an ovariectomized single rat. The percentage of acidophils was the same, but the percentage of basophils was 41 per cent lower in the parabiont. It is suggested that these facts are a reflection of the lower concentration of gonadotrophin (FSH) in the blood of the ovariectomized parabiont and therefore of a more rapid release of FSH and reversion of basophils to chromophobes. It is further suggested that a high FSH level in the blood retards release and thereby favors storage—a situation which exists in the ovariectomized single rat.

When 0.055 ug. per day of estradiol was injected into an ovariectomized parabiont the gonadotrophic activity of the pituitary gland of this rat was greater than that of the pituitary of an uninjected ovariectomized parabiont or of an ovariectomized single rat, and the percentage of basophils was less and the percentage of acidophils was greater. Under these conditions it is suggested that estrogen, in the small amounts administered, increases the number of acidophils by accelerating mitotic activity in these cells (Baker and Everett, 1944), and/or by conversion of chromophobes into acidophils. (For additional evidence supporting the concept that estrogen and also testosterone increase the number of acidophils and that LH is produced by the acidophils, see Kraus, 1933; Friedgood and Dawson, 1938, 1940; Wolfe, 1941; Wainman, Reese and Koneff, 1942; and Finerty, 1942.) Severinghaus (1939) has adequately summarized the literature and correlated the cytological and physiological evidence regarding the effects of gonadectomy and the administration of estrogens on the pituitary gland.

Estradiol administered to an ovariectomized single animal had the same effect as when administered to an ovariectomized parabiont, i.e., the gonadotrophic activity and percentage of acidophils were increased, and the percentage of basophils was decreased when compared to an ovariectomized single rat.

It should be noted that the gonadotrophic activity of the pituitary gland of a single ovariectomized rat given estradiol was considerably greater than that of an ovariectomized estrogen-treated parabiont, although the percentage of chromophils was approximately the same

in both animals. This may be the result of a higher level of LH of the ovariectomized single animal as compared to that in the blood of the parabiotic rats, thereby causing the storage of more LH in the acidophils of the single animal.

Increased gonadotrophic content of the pituitary in response to estrogen treatment is an effect that has not been generally reported. The difference in results may be attributed to the small dose of estrogen used in our studies. Baker and Everett (1944), who used small quantities of diethylstilbestrol for 4 or 7 days, found an increased mitotic activity, hypertrophy of the Golgi apparatus and increase in relative and absolute number of acidophils, and degranulation and reduction in the number of basophils. Continuation of treatment with their maximum dosage for 14 days resulted in slight reduction in the number of acidophils. Meites and Turner (1942) have also reported that the lactogen content of the hypophysis is increased more by small doses of diethylstilbesterol than by larger doses.

On the basis of the data presented in this paper a tentative explanation of pituitary-ovarian interaction in normal and parabiotic female rats is offered, the essential features of which are as follows:

FSH is produced by the basophils and LH by the acidophils (Severinghaus, 1939). Increasing quantities of blood estrogen decrease the rate of differentiation of chromophobes into basophils and inhibit the formation of FSH, but accelerate the conversion of chromophobes into acidophils and the production of LH. High blood estrogen for prolonged periods of time, however, inhibits the differentiation of chromophobes into chromophils and the formation of FSH and LH (Wolfe, 1935, a,b).

The rate at which basophils and acidophils give off their secretion and revert to chromophobes is dependent upon the level of FSH and LH in the blood, which in turn is controlled by inactivation and ovarian utilization. High blood levels of FSH or LH result in storage in the respective chromophils producing them; low levels result in release.

Since estrogen regulates the production of gonadotrophins by the chromophils and the level of the gonadotrophins in the blood regulates their storage, it follows that varying quantities of FSH and LH may be found in the pituitary gland at any one time. Therefore, the gonadotrophic potency of the pituitary gland, as determined by ovarian weight changes in immature rats, is dependent upon the ratio of FSH to LH and the absolute quantities of each.

SUMMARY

The pituitary gland of ovariectomized female rats in parabosis with a normal female has a greater gonadotrophic activity and is more basophilic than the pituitary of a single intact female, but has less activity and fewer basophils than a single ovariectomized female. The

pituitary gland of the intact parabiont in which excessive ovarian hypertrophy occurs has the lowest gonadotrophic activity and the smallest number of chromophils.

Injection of small doses of estradiol reduces the percentage of basophils in the pituitary gland of ovariectomized parabiotic and single rats, and at the same time increases the number of acidophils. The gonadotrophic content of the pituitary glands of these rats is greater than that of similar non-injected animals.

These observations provide the basis for the following hypotheses: 1) FSH is produced by the basophils and LH by the acidophils of the pituitary gland, 2) the differentiation of each type of chromophil from chromophobes is controlled by the level of blood estrogen, and 3) the rate at which the chromophils release their gonadotrophin is dependent upon the level of blood FSH and LH.

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THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE ON THE URINARY EXCRETION OF SODIUM, CHLORIDE, POTASSIUM, NITROGEN AND GLUCOSE IN NORMAL RATS

DWIGHT J. INGLE, CHOH HAO LI AND HERBERT M. EVANS
From the Research Laboratories, The Upjohn Company
KALAMAZOO, MICHIGAN
AND
The Institute of Experimental Biology, University of California
BERKELEY, CALIFORNIA

THE PRODUCTION of glycosuria and hyperglycemia in normal force-fed rats by the administration of large amounts of either 17-hydroxycorticosterone or of 17-hydroxy-11-dehydrocorticosterone has been reported (Ingle, Sheppard, Evans and Kuizenga, 1945). In the present study it was shown that the administration of pure adrenocorticotrophic hormone to similar rats force-fed a high carbohydrate diet also caused glycosuria and hyperglycemia, with an accompanying increase in the excretion of nitrogen and potassium and inhibition of growth. It was not established that the hormone had any direct effect on the excretion of sodium and chloride. Some of the data relative to production of glycosuria have been the subject of a preliminary report (Ingle, Winter, Li and Evans, 1945).

METHODS

Male rats of the Sprague-Dawley strain were maintained on a diet of Purina dog chow until they reached a weight of approximately 300 gm. They were then placed in metabolism cages and maintained on a fluid diet administered by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technique of force-feeding and the high carbohydrate diet used were modifications of those described by Reinecke, Ball and Samuels (1939). The diet was made according to Table 1. During the period of adaptation to force-feeding, the amount of diet was increased gradually to prevent the development of "food-shock." The animals were brought to a full feeding of 26 cc. per rat per day on the fifth day. This volume of diet contained approximately 15 gm. of available carbohydrate.

The animals were housed in an air-conditioned room in which the temperature was maintained at 74 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. Twenty-four-hour samples of urine were collected at the same hour each day and were preserved with thymol and 1 gm. per

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sample of citric acid to insure the acidity of the samples for nitrogen analysis. The following methods of analysis were used: blood glucose, Miller and Van Slyke (1936); urinary glucose, Benedict (1911); urinary chloride, Schales and Schales (1941) and urinary sodium and potassium by the flame photometer, Barnes, Richardson, Berry and Hood (1945). The determination of non-protein nitrogen was by the micro-Kjeldahl procedure as follows; proteins were precipitated as salts of tungstic acid by the Folin-Wu procedure; the organic matter was oxidized by sulfuric acid and hydrogen peroxide; the ammonia was distilled off into a standard acid solution and titrated with standard base.

TABLE 1

Constituent	Grams
Cellu flour (Chicago Dietetic Supply)	120
Osborne and Mendel salt mixture	40
Wheat germ oil	10
Mazola oil	10
Cod liver oil	10
Vitamin K (2-methyl-1, 4-naphthoquinone)	100 mgm.
Dried yeast (Pabst)	100
Egg albumin (Merck)	160
Starch	500
Dextrin	250
Sucrose	250
Water to make total of	2000 cc.

Adrenal demedullation was by the procedure described by Ingle and Griffith (1942) at a body-weight of 150–160 gm. Asepsis was successfully maintained in these operations. The animals were placed in the metabolism cages when they reached a weight of 300 gm.

The adrenocorticotrophic hormone (ACTH) was prepared by the procedure described by Li, Evans and Simpson (1943). Following control periods of 10 days, the animals were given adrenocorticotrophic hormone (ACTH) by subcutaneous injection in divided doses every 2 hours for a total of 7 injections per day.

EXPERIMENTS AND RESULTS

Experimental. Following a control period of 10 days, normal rat 1701 (Figure 1) was given 3 mgm. of ACTH per day for 11 days but exhibited no glycosuria during this period. The dosage was then increased to 7 mgm. of ACTH per day for 4 days. The animal excreted urinary glucose during the last 2 days and was killed for necropsy at the end of this period.

On the basis of the results obtained with animal 1701, 5 normal rats which had been observed during a control period of 10 days were given 7 mgm. of ACTH per day for 10 days. At the end of this period, rats 2002 and 2101 (Figures 2 and 3) were killed for necropsy. Rats 1406, 1703 and 2003 (Figures 4, 5 and 6) were observed for 7 days following withdrawal of the hormone and were then killed for necropsy.

In order to test the possible role of the adrenal medulla in causing the glycosuria during treatment with ACTH, 3 adrenal-demedullated

rats (Figure 7) were given 7 mgm. of ACTH per day for 6 days and were then killed for necropsy. Because of the limited quantity of the hormone, it was not practical to study a larger series of animals or to continue the injections for a longer period. As a further test of the possibility that the glycosuria caused by ACTH in normal animals

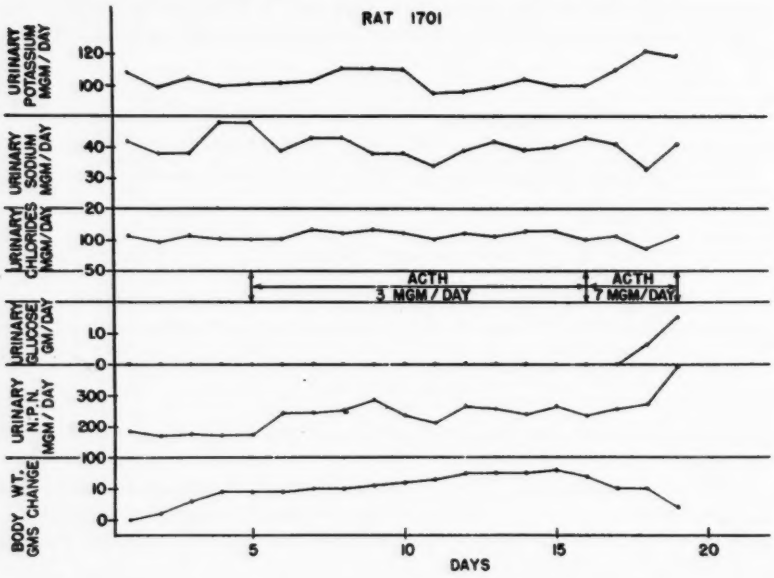

FIG. 1. Intact rat 1701. (In this and each of the other figures a change in the administration of a test substance is plotted on a vertical line with the data obtained at the end of the 24-hour period within which the change was made.)

was due to glycogenolysis mediated by a response of the adrenal medulla, the hormone was tested for its immediate effect on the level of blood glucose in the following manner. In 6 normal force-fed rats the glucose of tail blood was determined just prior to and 20 minutes following the injection of 1 mgm. of ACTH, which was the quantity of each single dose in the above experiments. The animals were tested following a 4-hour fast and a 16-hour fast.

Urinary glucose. All of the rats were free from glycosuria during the control periods. Rat 1701 (Figure 1) failed to excrete glucose during the administration of 3 mgm. of ACTH per day but did excrete glucose when the dosage was increased to 7 mgm. per day. Four of the 5 rats which were given 7 mgm. of ACTH per day for 10 days excreted amounts of glucose up to a maximum of over 5 gm. per day (Figure 3). Rat 1703 (Figure 5) failed to develop glycosuria. Following withdrawal of the hormone, the glycosuria disappeared from the 2 rats (Figures 4 and 6) which had excreted glucose during the injection

period. Among the 3 adrenal-demedullated rats (Figure 7), 1 animal excreted small amounts of glucose for 2 days and the other 2 animals remained free from glycosuria.

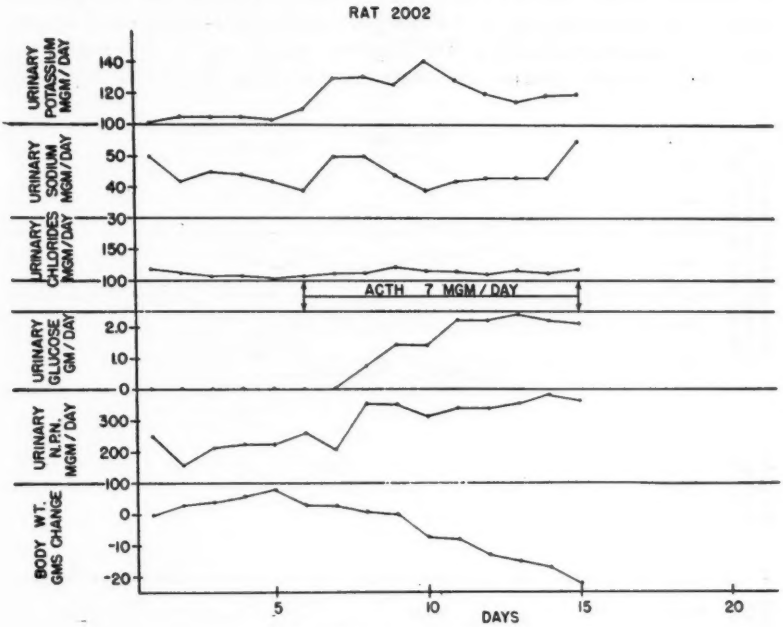

FIG. 2. Intact rat 2002.

Blood glucose. Data which illustrate the changes in blood glucose as related to the administration of ACTH are summarized in Table 2. Once during each phase of the experiment samples of blood were taken from the tail just prior to the morning feeding (16-hour fast) and at

TABLE 2. VALUES FOR BLOOD GLUCOSE

Rat	Experimental condition	ACTH daily dose mgm.	Values for blood glucose mgm. per cent						
			16-hour fast	Hours following morning feeding				Hours following evening feeding	
				2	4	6	7	3½	5
2002	Intact	0	88	118	110	127	109	125	129
2002	Intact	7	85	120	181	202	211	298	304
2101	Intact	7	103	151	195	283	265	464	235
1407	Adrenal Demedullated	7	100	105	109	107	131	181	162
1008	Adrenal Demedullated	7	87	124	116	119	103	133	146

2, 4, 6 and 7 hours following the morning feeding. Some additional samples were taken following the evening feeding. All of the normal animals tolerated the high carbohydrate diet without the development of hyperglycemia during the control periods. During the administration of the hormone, all of the unoperated animals developed hyperglycemia following feeding. The highest values, up to 464 mgm. per cent, were recorded following the evening feeding. Rat 1703 (Figure 5)

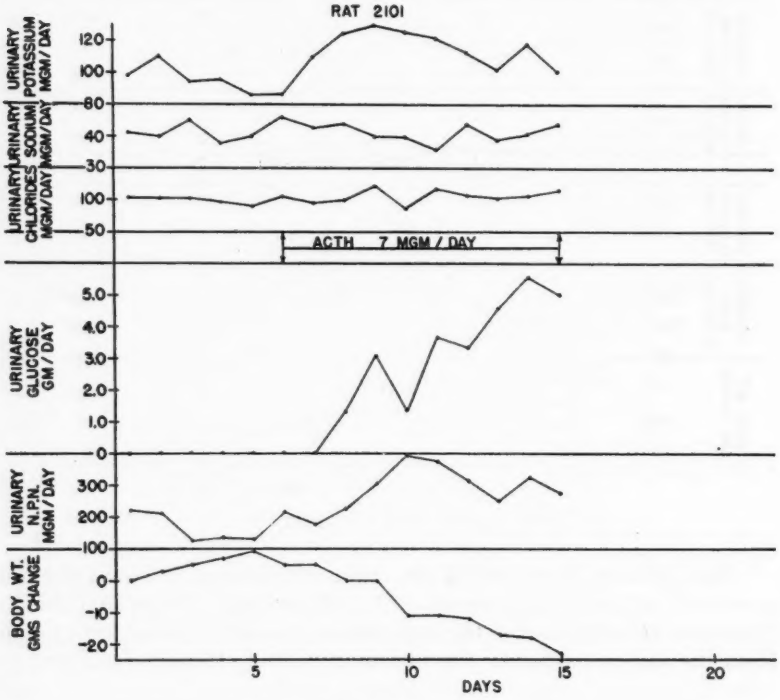

FIG. 3. Intact rat 2101.

developed hyperglycemia up to 315 mgm. per cent following feeding, although it did not excrete any significant amount of urinary glucose. The values during fasting ranged from 74 to 143 mgm. per cent. Among the 3 adrenal-demedullated rats treated with ACTH, the highest value recorded following feeding was 181 mgm. per cent.

The small range of rise in the level of blood glucose following the injection of 1 mgm. of ACTH (Table 3) was similar to that which followed the injection of saline, thereby indicating that rapid and extensive glycogenolysis does not follow the injection of ACTH.

TABLE 3. VALUES FOR BLOOD GLUCOSE IN NORMAL FORCE-FED MALE RATS BEFORE AND AFTER INJECTIONS OF 1 UNIT OF ACTH AND OF CONTROL INJECTIONS OF SALINE

Rat	Fasting				4 Hours post-feeding			
	ACTH		Saline		ACTH		Saline	
	0	20 min.	0	20 min.	0	20 min.	0	20 min.
310	102	114	127	105	109	112	117	126
111	108	93	96	111	114	117	117	99
112	96	111	87	99	103	112	108	118
113	96	111	96	112	115	117	114	115
114	97	127	94	96	108	118	108	121
115	91	94	102	114	115	120	108	117
Avg.	98	108	100	106	111	116	112	116

Body-weight. The unoperated rats each gained weight during the control periods and lost weight during the administration of ACTH. The effect of ACTH on the weights of the adrenal-demedullated rats was much less striking.

Urinary non-protein nitrogen. The unoperated rats each showed a marked rise in the level of urinary nitrogen during the administration of ACTH. In these experiments the numbers of rats were not sufficiently large and the period of injection not sufficiently long to es-

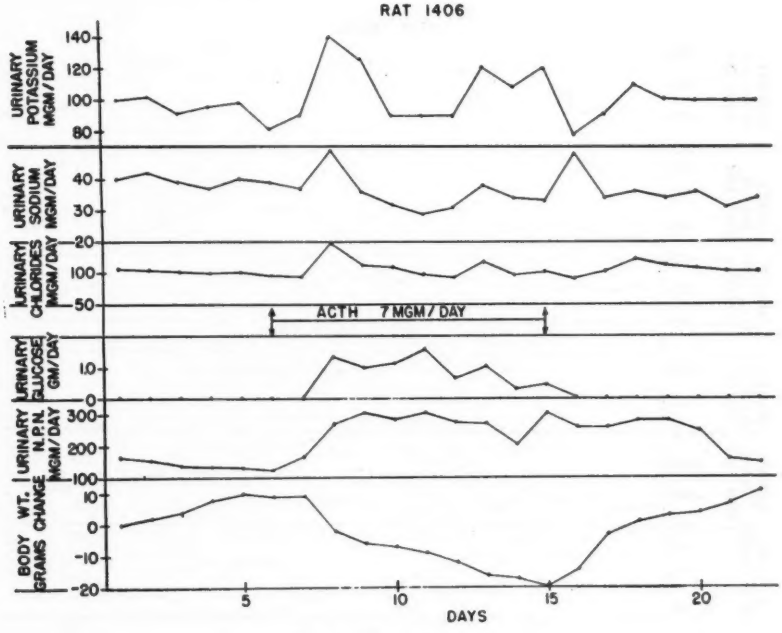

FIG. 4. Intact rat 1406.

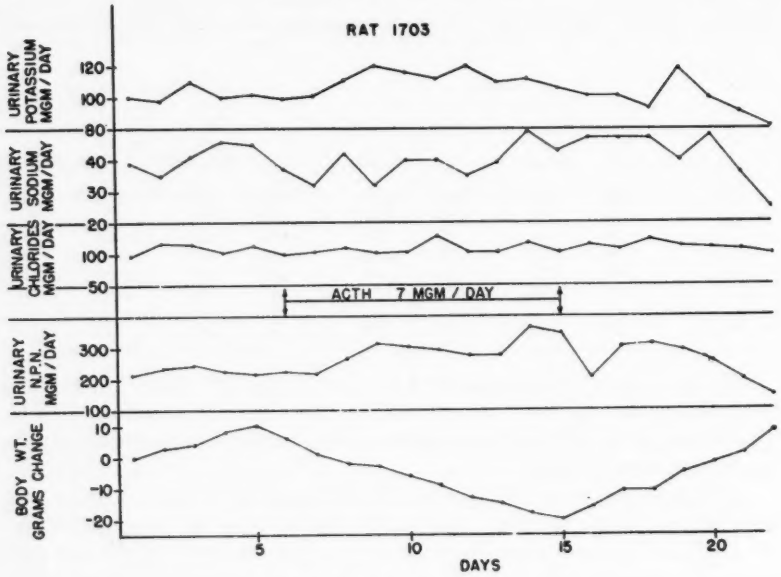

FIG. 5. Intact rat 1703.

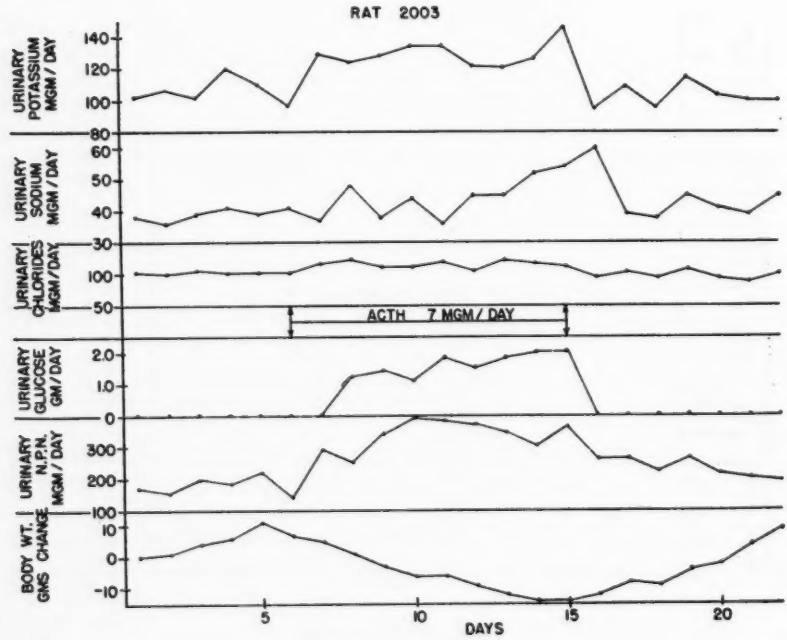

FIG. 6. Intact rat 2003.

tablish whether or not the rise is sustained. The effect of ACTH on the nitrogen balance of the adrenal-demedullated rats was much less striking.

Urinary potassium. The changes in urinary potassium tended to parallel the changes in urinary nitrogen during the administration of ACTH. A rise in the level of urinary potassium accompanied the rise in urinary nitrogen and the loss of weight. These changes were reversed when the injection of ACTH was stopped in rats 1406 and 2003.

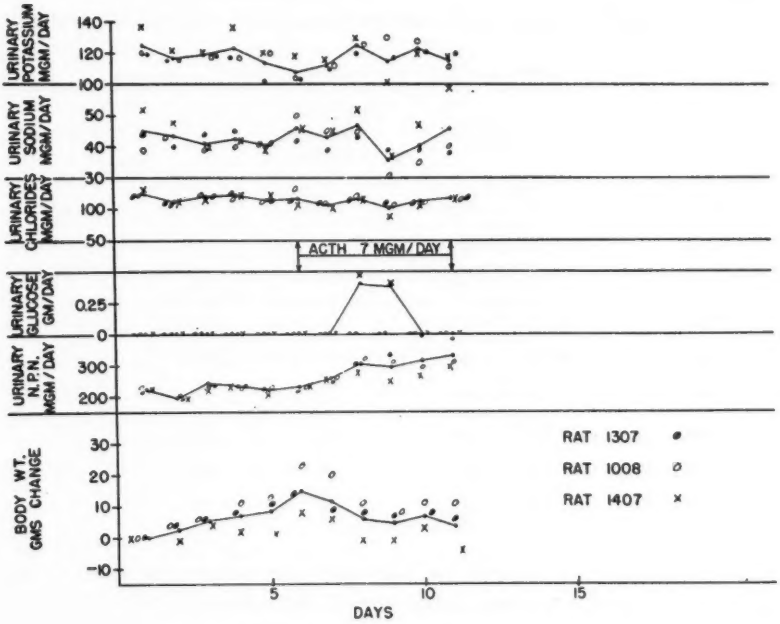

FIG. 7. Adrenal demedullated rats.

Urinary sodium and chloride. In these experiments it was not established that this dosage level of ACTH affected the excretion of sodium and chloride. In 2 of the animals (Figures 3 and 6) the onset of glycosuria was accompanied by an increase in the excretion of sodium and chloride. In rats 1406 and 2003 (Figures 3 and 6) the withdrawal of ACTH was followed by a temporary increase in the excretion of sodium which may have been related to the accompanying retention of potassium.

Necropsy data. The weights of the adrenal and thymus glands are recorded in Table 4. The marked hypertrophy of the adrenal glands and the almost complete atrophy of the thymus tissue is indicated by comparing these values with an average adrenal weight of 36 mgm.

and an average thymus weight of 410 mgm. for 6 control rats of similar weight which were force-fed the same diet. In rats 1406 and 2003, which were observed for 7 days following the withdrawal of ACTH, the adrenal and thymus weights had changed towards normal. In the 3 adrenal-demedullated animals the regenerated enucleated glands had approximately doubled in size over those of similar animals not treated with ACTH but they were still very small as compared to intact adrenals stimulated by ACTH. Likewise, the regression of the thymus was marked but less complete than observed in normal animals.

In rat 1703 an ulcer was found in the pyloric end of the stomach.

TABLE 4. ORGAN WEIGHTS AT NECROPSY

Rat	Experimental condition	Days after stopping ACTH	Adrenals pair	Thymus
2002	Intact ACTH 10 days	0	131	28
2101	Intact ACTH 10 days	0	146	27
1701	Intact ACTH 13 days	0	125	37
1406	Intact ACTH 10 days	7	122	117
1703	Intact ACTH 10 days	7	80	175
2003	Intact ACTH 10 days	7	81	149
1008	Demedullated ACTH 7 days	0	45	131
1307	Demedullated ACTH 7 days	0	54	76
1407	Demedullated ACTH 7 days	0	51	68

In each animal treated with ACTH there was some regression in size of the testes and a marked regression of the seminal vesicles.

DISCUSSION

The metabolic changes induced by ACTH were like those caused by 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone in similar experiments (Ingle, Sheppard, Evans and Kuizenga, 1945). It is significant that a diabetic state was induced in normal animals by stimulating the adrenal cortices to secrete excessive amounts of its own hormones. In the experiments with the C-11, 17-oxygenated adrenal steroids, glycosuria was induced by the administration of 5 mgm. per day of 17-hydroxy-11-dehydrocorticosterone. In the work test (Ingle, 1944), this compound was found to have the activity equivalent of approximately 200 gm. of beef adrenal glands or of 5 cc. of cortical extract per 1 mgm. On such a basis it can be estimated that the adrenal glands of the rats in the present experiments were stimu-

lated to secrete the activity equivalent of 20 to 25 cc. of beef adrenal extract (40 gm. of gland per 1 cc.) per day. Some fallacies may be involved in this estimation, but there is much supporting evidence that the secretory activity of normal adrenal glands is very large (Ingle, 1944).

Many drugs and toxins cause hyperglycemia and temporary glycosuria by means of rapid and extensive glycogenolysis in the liver and peripheral tissues, presumably by stimulation of the adrenal medulla to release epinephrine into the blood. In these experiments it was considered that ACTH may have caused glycosuria in such a non-specific manner. Although the number of adrenal-demedullated rats studied was too small to permit definite conclusions, the failure of ACTH to cause a more marked glycosuria in any of the 3 adrenal-demedullated rats might be used as evidence that the medulla played an essential role in the response of normal animals to ACTH. To the contrary, we favor the interpretation that the medulla is unessential for the response to ACTH and that the demedullated adrenal, although capable of hypertrophy and hyperfunction in response to ACTH, may have a maximum secretory response which is less than that of the intact adrenal. Simpson, Li and Evans (1945) have shown that the cortex of the demedullated adrenal is responsive to ACTH, and hypertrophy was observed in these experiments; but the extent of hypertrophy, thymus atrophy, and metabolic changes was less than for normal animals. When ACTH was tested in the normal animal it did not cause hyperglycemia immediately following injection, as would be expected if it caused glycogenolysis. Moreover, very high values for blood glucose were recorded 6 hours following the injection of ACTH in the diabetic animals of these experiments. Hyperglycemia due to epinephrine or other drugs and toxins is of short duration. The delay in onset of glycosuria is characteristic of true diabetogenic agents, not of glycogenolytic drugs, and the extent of glycosuria was much greater than has been found to be produced by glycogenolytic agents.

From the metabolic changes produced by ACTH in these and other experiments, it can be assumed that this hormone stimulates the secretion of adrenal steroids which affect organic metabolism, such as the C-11-oxygenated compounds. Does ACTH stimulate the secretion of compounds which cause the renal retention of sodium and chloride as does the synthetic compound, 11-desoxycorticosterone? ACTH has not been proven to cause sodium retention in any of our experiments. It is possible that an increased secretion of sodium-retaining compounds would be masked by secretion of the C-11, 17-oxygenated compounds which have the property (perhaps non-specific) of causing a temporary increase in the excretion of sodium and chloride. The hypophysectomized animal does not lose vital amounts of sodium and chloride as does the adrenalectomized animal, although

it exhibits deficiencies in organic metabolism due in part to the secondary adrenal cortical insufficiency. The secretion of highly active sodium-retaining steroids by the adrenal cortex, if it normally secretes such compounds at all, may be independent of ACTH. This possibility has been suggested by a number of other investigators.

SUMMARY

Male rats of approximately 300 gm. initial weight were force-fed a high carbohydrate diet. Glycosuria and hyperglycemia were induced in 6 out of 7 normal rats by the administration of 7 mgm. per day of pure adrenocorticotrophic hormone. There was an accompanying loss of weight, increased excretion of urinary non-protein nitrogen and of potassium. There were no consistent changes in the excretion of sodium and of chloride. The hypertrophy of the adrenal cortices and the atrophy of the thymus were very marked. The experiments were repeated in 3 adrenal-demedullated rats. A mild glycosuria appeared in 1 animal. The other changes were much less striking than in intact animals.

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THE PRODUCTION OF ALIMENTARY GLYCOSURIA BY FORCED FEEDING IN THE RAT

DWIGHT J. INGLE

From the Research Laboratories, The Upjohn Company

KALAMAZOO, MICHIGAN

FIFTY-SEVEN YEARS AGO Hofmeister (1889) called attention to the limited ability of the normal animal to assimilate orally-administered carbohydrate, and demonstrated that glucose is excreted in the urine when the assimilation limit is exceeded. The tolerance limits of normal animals to orally-administered carbohydrate, when time is permitted for adaptation to overfeeding, have not been thoroughly studied.

In these experiments normal rats were force-fed a high carbohydrate diet with different rates of increment until death. Very large amounts of diet were tolerated by each rat, but after the limit of tolerance was exceeded each rat excreted significant amounts of urinary glucose and developed hyperglycemia following feeding. The results of a preliminary study have been reported (Ingle, 1945[b]).

METHODS

Male rats of the Sprague-Dawley strain were maintained on Purina Dog Chow until they reached a weight of 300 gm. They were then placed in

TABLE 1

Constituent	Grams
Cellu flour (Chicago Dietetic Supply)	120
Osborne and Mendel salt mixture	40
Wheat germ oil	10
Mazola oil	10
Cod liver oil	10
Vitamin K (2-methyl-1, 4-naphthoquinone)	100 mg.
Dried yeast (Pabst)	100
Egg albumin (Merck)	160
Starch	500
Dextrin	250
Sucrose	250
Water to make total of	2000 cc.

metabolism cages and maintained on a fluid diet administered by stomach tube each morning (8:30 to 9:00 A.M.) and late afternoon (4:30 to 5:00 P.M.). The techniques and diet were modifications of those described by Reinecke, Ball and Samuels (1939). The diet was made according to Table 1. The addition of Vitamin K (2-methyl-1, 4-naphthoquinone) to the diet ef-

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fectively prevented the occurrence of the fatal diffuse hemorrhages which were observed in a preliminary study (Ingle, 1945[b]). During the period of adaptation to forced feeding, the amount of diet was increased gradually to prevent the development of "food-shock." The animals were brought to a feeding of 26 cc. per rat per day on the fifth day and were maintained at this level, which represents a normal caloric intake for rats of this weight, for seven days. Thereafter, the amount was increased at a certain rate per day (see Experiments and Results) until the death of the animal.

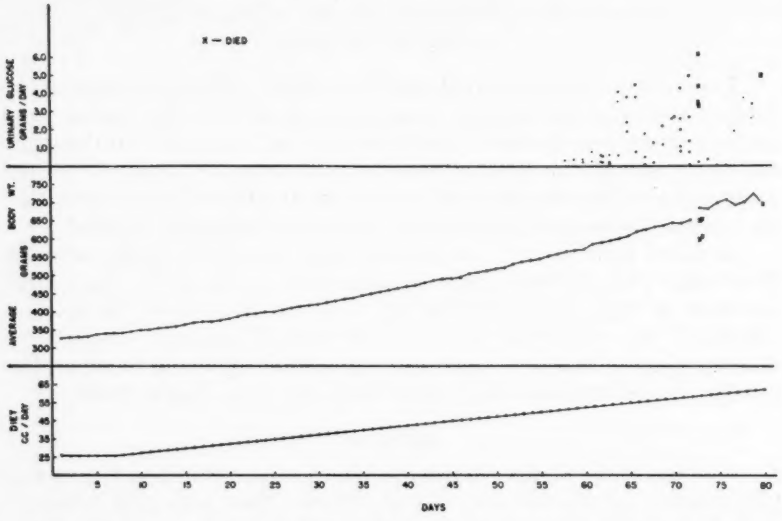

FIG. 1. Individual values for urinary glucose in 5 normal rats overfed by an increment of 0.5 cc. of diet per day until death.

In this and each of the other figures an increase in the amount of diet is plotted on a vertical line with the data obtained at the end of the 24-hour period within which the change was made. The average body-weight is shown until the time of death of the first animal. Thereafter, the individual values for body-weight are plotted.

The animals were housed in an air-conditioned room in which the temperature was maintained at 74 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 A.M.) and were preserved with thymol. Blood glucose was determined by the method of Miller and Van Slyke (1936), and urine glucose by the method of Benedict (1911).

EXPERIMENTS AND RESULTS

Experimental. In Experiment 1 (Figure 1) five normal rats were overfed by an increment of 0.5 cc. per day; in Experiment 2 (Figure 2) six normal rats were overfed by an increment of 1 cc. per day; in Experiment 3 (Figure 3) six normal rats were overfed by an increment of 2 cc. per day; in Experiment 4 (Figure 4) six normal rats were over-

fed by an increment of 4 cc. per day and in Experiment 5 (Figure 5) six normal rats were overfed by an increment of 6 cc. per day.

In Experiment 6 (Table 2) single increments of food-intake were made in four groups of rats having six rats in each group. The amounts of diet per day were increased above the basal intake of 26 cc. to levels

TABLE 2. AMOUNTS OF GLUCOSE EXCRETED BY NORMAL FORCE-FED RAT FOLLOWING AN INCREASE ABOVE A NORMAL CALORIC INTAKE (26 CC. PER DAY)

Diet cc./day	Rats					
	106	207	306	406	506	607
34	0	0	0	0	0	0
34	0	0	0	0	0	0
34	0	0	0	0	0	0
34	0	0	0	0	0	0
34	0	0	0	0	0	0
	107	208	307	407	507	608
36	0	0	0	0	0	0
36	0	0	0	0	0	0
36	757	0	0	0	0	0
36	0	0	0	0	0	0
36	0	0	0	0	0	0
	108	209	308	408	508	609
38	0	0	0	0	0	0
38	694	0	100	0	216	0
38	2775	0	0	0	0	0
38	2085	0	0	0	0	0
38	535	0	0	0	0	0
38	0	0	0	0	0	0
40	0	0	0	0	0	0
40	252	99	212	1046	78	195
40	66	75	1612	61	200	264
40	0	0	218	0	0	0
40	0	0	0	0	0	0
40	0	0	0	0	0	0

of 34, 36, 38 and 40 cc. per day and were kept constant for periods of five or six days, at which time the diet was withdrawn.

Urinary glucose. All of the rats were free from glycosuria during the control periods. When the intake of food was increased gradually, all of the animals tolerated very large amounts before alimentary glycosuria developed. Within the range of increment of 0.5 cc. to 6 cc. of diet per day the amounts of diet tolerated by each group of rats were approximately the same, although there were marked differences in body-weight by the time the limit of tolerance was reached. The longest period that any rat had glycosuria prior to death was sixteen days. The maximum amount of glucose excreted by any rat was 10.525 gm. per day (Figure 2).

In Experiment 6 (Table 2) all six animals tolerated a one-step

increase of from 26 cc. to 34 cc. per day of the diet without the excretion of glucose into the urine. With increases to 36 cc. and 38 cc. one and three rats respectively showed a mild temporary glycosuria. With an increase to 40 cc. of diet per day all six rats excreted urinary glucose for two or three days and then the glycosuria disappeared in each animal.

Blood glucose. A systematic study of the changes in blood glucose

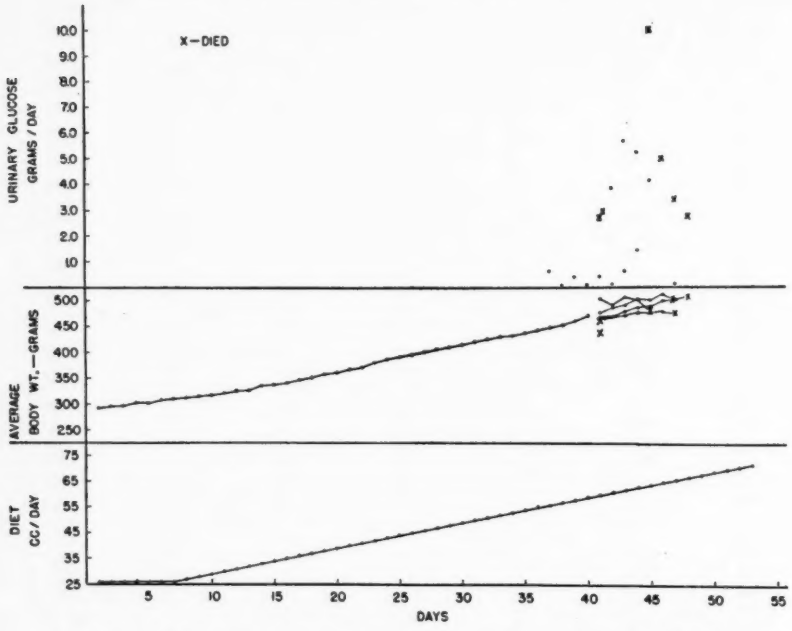

FIG. 2. Individual values for urinary glucose in 6 normal rats overfed by an increment of 1 cc. of diet per day.

was not carried out in these experiments. The analysis of blood samples from each animal established the fact that the animals were hyperglycemic during the time (post-feeding) that they excreted glucose. Values for blood glucose ranging from 267 to 352 were recorded. The values for blood glucose taken at the end of the sixteen-hour fast (between the afternoon and following morning feedings) were within normal limits.

Necropsy. Only a few gross pathologic changes were observed. The adrenal cortices were uniformly a dark red color and were enlarged. In the animals of Experiments 1 and 2, the range in weight for pairs of adrenal glands was 61 to 128 mg., with an average of 81 mg. The thymus was small in all cases, having a range in weight of 55 to 225 mg.,

with an average of 171 mg. The seminal vesicles were atrophic and the testes were soft and flabby. The range in weight for pairs of testes was 2268 to 3033 mg., with an average of 2515 mg. Since these overfed rats were heavier than normal adult rats, it is not possible to control the relationship of both age and weight to the size of organs. Twelve male rats which were approximately three months older than the overfed rats had an average body-weight of 493 gm. and average weights

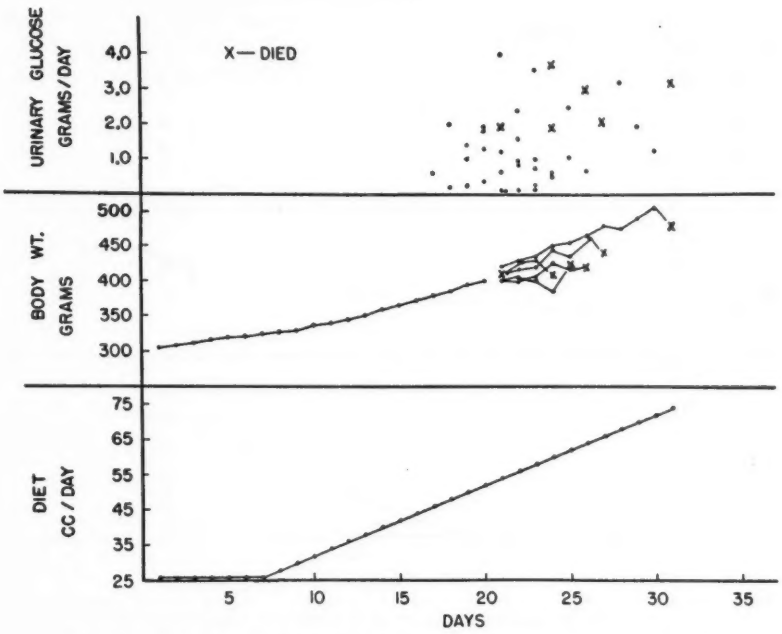

FIG. 3. Individual values for urinary glucose in 6 normal rats overfed by an increment of 2 cc. of diet per day.

of pairs of adrenals, testes and of the thymus of 55 mg., 3325 mg. and 410 mg. respectively.

No hemorrhages were observed in these animals. The animals which were force-fed for periods of forty days and longer were very obese at the time of death. In animals fed for seventy-three days and longer (Experiments 1 and 2) the body-length was greater than that of adult animals of this strain, thereby indicating that there was an increase in skeletal growth in these overfed animals.

In the five rats which were overfed by an increment of 0.5 cc. per day (Experiment 1) the volume capacity of the stomach was determined at necropsy to range between 100 cc. and 130 cc. per rat.

DISCUSSION

It is the opinion of the writer that these data can be related to the question of the general nature of the metabolic defect in pancreatic diabetes; i.e., is the glycosuria due principally to "under-utilization" or to "over-production" of carbohydrate? Very large amounts of the high carbohydrate diet were tolerated before the limit of tolerance was exceeded. On the basis of the amounts of urinary nitrogen ex-

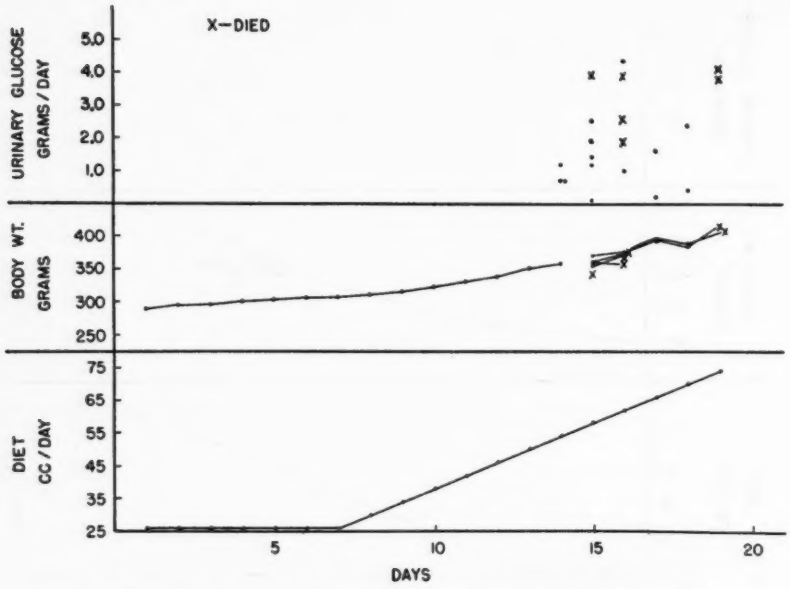

FIG. 4. Individual values for urinary glucose in 6 normal rats overfed by an increment of 4 cc. of diet per day.

creted by some of the animals and by making generally accepted assumptions regarding the extent of conversion of protein and the glycerol of fat to carbohydrate, it was estimated that the rat (wt. =535 gm.) from Experiment 1 (increment of 0.5 cc. of diet per day) with the lowest tolerance removed from its blood approximately 29.3 gm. of glucose in twenty-four hours, or an average of 228 mg. per 100 gm. of rat per hour, before glucose was excreted in the urine. Similarly, in Experiment 4 (increment of 4 cc. of diet per day) it was estimated that the rat (wt.=353 gm.) with the lowest tolerance removed from its blood 28.75 gm. of glucose in twenty-four hours, or an average of 339 mg. of glucose per 100 gm. of rat per hour, before glucose appeared in the urine. Since it was found that the values for blood glucose had returned to normal prior to the morning feeding, it can be

assumed that the maximum rate at which glucose was removed from the blood during the immediate post-feeding periods was even greater. If it is assumed that the ability of the diabetic animal to utilize (oxidation, storage and conversion) carbohydrate is normal, it must then be imagined that overproduction of glucose is sufficiently great to exceed the wide margin of tolerance which lies between the normal caloric intake and the assimilation limit and, in addition, to account for the excretion of such amounts of urinary glucose as are seen in pancreatic diabetes.

Some of the support for the hypothesis that the diabetic animal utilizes glucose to a normal extent is derived from the experiments of

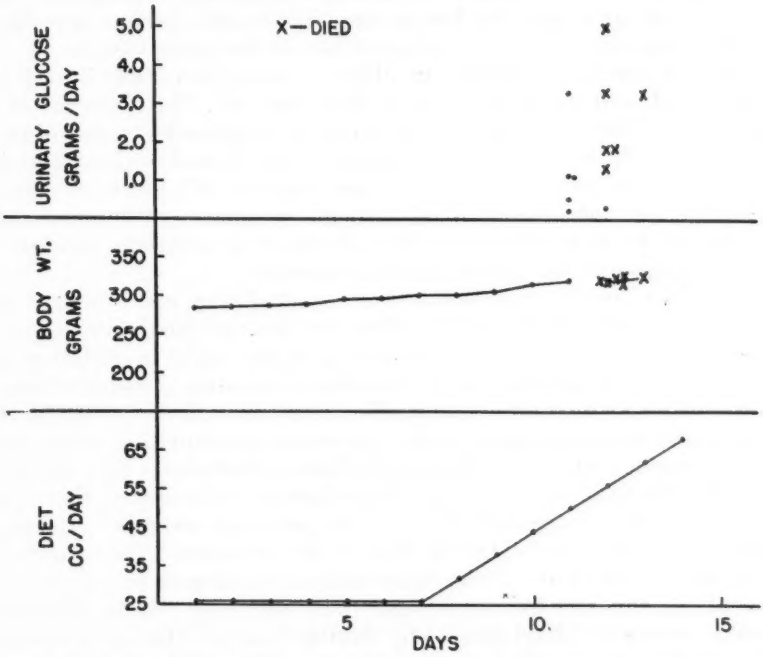

FIG. 5. Individual values for urinary glucose in 6 normal rats overfed by an increment of 6 cc. of diet per day.

Greeley and Drury (1940) and of Soskin and Levine (1937) on the utilization of glucose by the liverless rabbit and dog with and without the presence of the pancreas. The assumption that neither the removal of the liver nor a brief fast significantly alters normal tolerance to carbohydrate is erroneous. Inspection of the data from the above-mentioned experiments shows that the amounts of glucose tolerated were very low—actually at a diabetic level rather than representative

of utilization by the normal active animal in which a maximum tolerance to carbohydrate has developed. In the experiments of Greeley and Drury (1940), the fasted, eviscerated, diabetic rabbit utilized 13.8 mg. of glucose per 100 gm. per hour, and similar animals having an intact pancreas utilized 12.5 mg. of glucose per 100 gm. per hour. In non-fasted animals the values were 19.8 mg. and 20.6 mg. per 100 gm. per hour respectively. In the experiments of Soskin and Lenine (1937), the dogs were eviscerated in the morning of the fourth day following the beginning of the fast. At a blood-sugar level of 285 mg. per cent, the utilization was 41 mg. per 100 gm. of non-diabetic eviscerated dog per hour. In unpublished experiments the writer has also found that the maximum amount of glucose utilized by the eviscerated rat is also very low as compared to the amounts of carbohydrate tolerated by normal adapted rats in the present study.

Under normal conditions the diabetic animal or patient is active, non-fasted, and possesses an intact liver and gut. The ability of the diabetic to tolerate carbohydrate should be appraised in terms of the voluntary caloric intake of the normal active animal or patient and, in addition, in terms of the maximum tolerance which can be developed by the normal individual through adaptation. Whatever the nature of the metabolic defect in diabetes, it is a chronic condition in which there is time for maximum adaptation.

The data of this experiment do not include any measurement of the amounts of carbohydrate oxidized by these overfed animals and, therefore, are not relative to the question of the extent of oxidation of carbohydrate in diabetes, since oxidation is only one phase of utilization. Although there was no quantitative estimation of the extent of conversion of carbohydrate to fat, the rapid accumulation of fat observed during the overfeeding of the high carbohydrate is in accord with the findings of many other investigators and suggests that the conversion of carbohydrate to fat is the principal means of utilizing the excess carbohydrate. On the basis of this assumption the following question can be raised: If the intact animal can dispose of a large excess of carbohydrate by converting it to fat, why does not the diabetic animal reduce its blood glucose by storing it as fat? This question is answered by the excellent studies of Stetten (1944, 1946), which have shown that the diabetic animal has a limited ability to convert carbohydrate to fat.

There may be some significance in the observation that animals caused to die by the overfeeding of a high carbohydrate diet show some hypertrophy of the adrenal cortices. Several years ago the writer (Ingle, 1942) raised the question as to whether the administration of an excess of carbohydrate would suppress the size of the cortex. As far as observations in this laboratory are concerned there is no close relationship between the size of the adrenals and diet (Ingle, 1945[a]).

The collateral changes in structure and chemistry of tissues which

accompany alimentary glycosuria and, in fact, the whole problem of the damaging effects of overfeeding remain to be investigated.

SUMMARY

Normal male rats were force-fed a high carbohydrate diet at different rates of increment until death. Very large amounts of carbohydrate were tolerated by each rat before the development of alimentary glycosuria. After the limit of tolerance was exceeded, each rat excreted significant amounts of urinary glucose. Within a wide range of increment in overfeeding, the level of tolerance was approximately the same for each group and was relatively independent of final bodyweight. The longest period that any rat had glycosuria prior to death was sixteen days. The maximum amount of glucose excreted by any rat in a twenty-four-hour period was 10.525 gm. It is pointed out that any explanation of the metabolic defect in pancreatic diabetes in animals, or diabetes mellitus in patients, must account for the loss in tolerance which lies between the normal caloric intake and the assimilation limit, as well as the excretion of such amounts of urinary glucose as are seen in diabetes.

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A COMPARISON OF THE ACUTE EFFECTS OF CORTICOSTERONE AND 17-HYDROXYCORTICOSTERONE ON BODY WEIGHT AND THE URINARY EXCRETION OF SODIUM, CHLORIDE, POTASSIUM, NITROGEN AND GLUCOSE IN THE NORMAL RAT

DWIGHT J. INGLE, RUTH SHEPPARD, ELIZABETH A. OBERLE AND MARVIN H. KUIZENGA

From the Research Laboratories, The Upjohn Company

KALAMAZOO, MICHIGAN

THE PURPOSE of this study was to further evaluate the differences in the biologic properties of corticosterone and its C-17-oxygenated derivative. At a dosage level of 5 mgm. per day, each compound caused glycosuria in some of the animals and an increase in the excretion of sodium, chloride, potassium and nitrogen. The 17-oxygenated compound caused the greatest response. At smaller doses 17-hydroxycorticosterone, but not corticosterone, caused an increased sodium excretion, and the former compound had a greater effect on weight and nitrogen balance.

METHODS

Infection-free male rats of the Sprague-Dawley strain were used. The stock diet was Purina Dog Chow. When the rats reached a weight of approxi-

TABLE 1

Constituent	grams
Cellu Flour (Chicago Dietetic Supply)	120
Osborne and Mendel salt mixture	40
Wheat germ oil	10
Mazola Oil	10
Cod liver oil	10
Vitamin K (2 methyl-1,4 naphthoquinone)	100 mgm.
Dried yeast (Pabst)	100
Egg albumin (Merck)	160
Starch	500
Dextrin	250
Sucrose	250
Water to make total of	2000 cc.

mately 300 gm. they were adapted to the forced feeding of a high carbohydrate diet administered by stomach tube each morning (8:30 to 9:15 A.M.)

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and afternoon (4:15 to 5:00 P.M.). The technique of forced feeding and the diet (Table 1) were modifications of those described by Reinecke, Ball and Samuels (1939). The rats were brought to a full feeding of 26 cc. per day on the fifth day. This amount of diet contained approximately 15 gm. of available carbohydrate.

At the beginning of forced feeding, the animals were transferred to metabolism cages in an air-conditioned room in which the temperature was

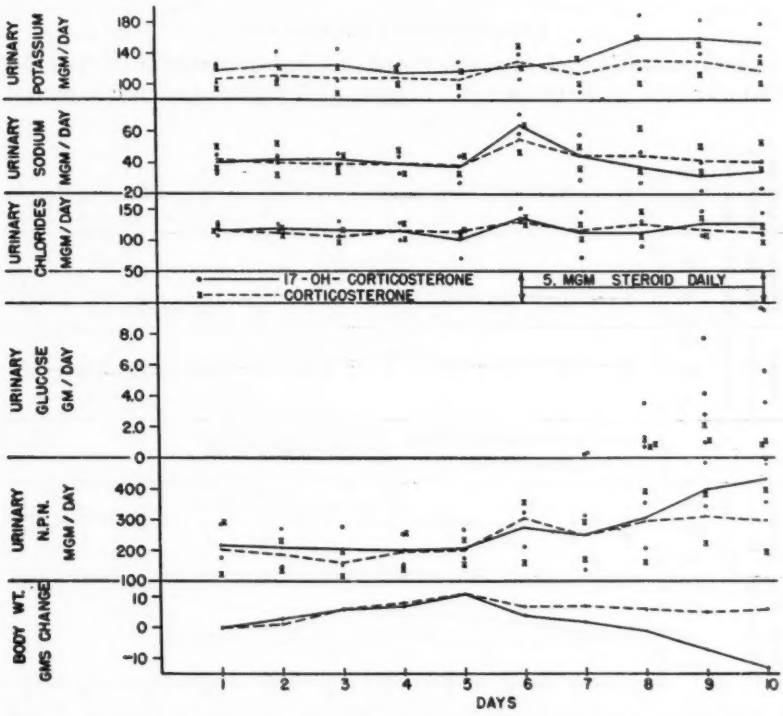

FIG. 1. Averages and range of values for 5 pairs of normal rats at a dosage level of 5 mgm. of adrenal cortical steroids per day. (In each figure a change in the administration of a test substance is plotted on a vertical line with the data obtained at the end of the 24-hour period within which the change was made.)

maintained at 74 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. Twenty-four-hour samples of urine were collected at the same hour each day (8:00 to 8:30 A.M.) and preserved with thymol and added citric acid (1 gm. per sample) to insure the acidity of the urines for nitrogen analysis. The following methods of analysis were used: blood glucose (Miller and Van Slyke, 1936); urinary glucose (Benedict, 1911); urinary chloride (Schales and Schales, 1941) and urinary sodium and potassium by the flame photometer (Barnes, Richardson, Berry and Hood, 1945). The determination of non-protein nitrogen was by the micro-Kjeldahl procedure as follows: pro-

teins were precipitated as salts of tungstic acid by the Folin-Wu procedure, the organic matter was oxidized by sulfuric acid and hydrogen peroxide and the ammonia was distilled off into a standard acid solution and titrated with standard base.

The adrenal cortical steroids were prepared as fine suspensions in sesame oil and were administered by subcutaneous injection in divided daily doses immediately following each feeding. The volume of oil was constant at ½ cc. per injection.

EXPERIMENTS AND RESULTS

Five rats (total of 40) were tested at 4 dosage levels of each of the two steroids. In Experiment 1 (Figure 1) 5 mgm. of each compound

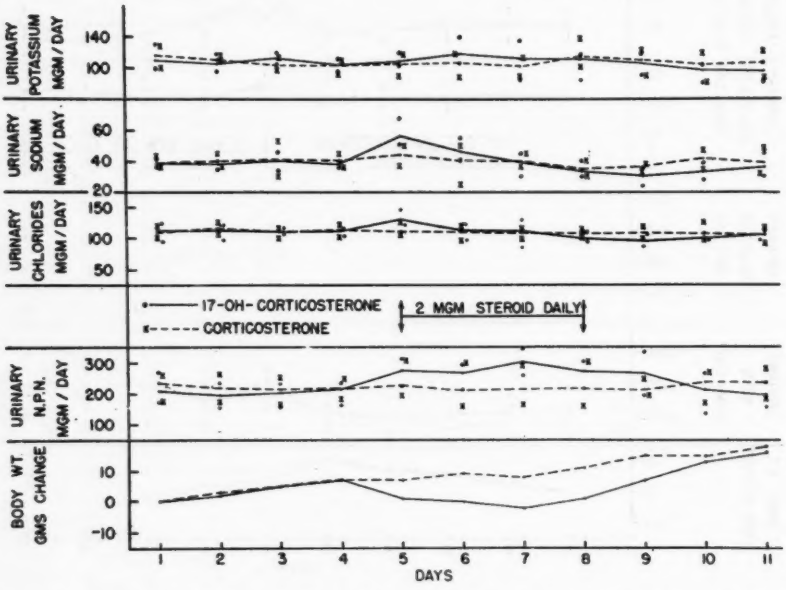

FIG. 2. Averages and range of values for 5 pairs of normal rats at a dosage level of 2 mgm. of adrenal cortical steroids per day.

were given per day for 5 days, at which time the experiment was terminated. In Experiment 2 (Figure 2) 2 mgm. of each compound were given per day for 4 days, and the experiment was terminated 4 days following withdrawal of the compound. In Experiment 3 (Figure 3) 1 mgm. of each compound was given per day for 4 days, and the experiment was terminated 4 days following withdrawal of the compound. In Experiment 4 (Figure 4) 0.5 mgm. of each compound was given per day for 4 days, and the experiment was terminated 4 days after the injections were stopped.

Urinary glucose. All of the rats were free from glycosuria during

the control periods. During the injection of 5 mgm. per day of the steroids, 4 rats receiving 17-hydroxycorticosterone developed glycosuria up to a maximum value of over 9 gm. of glucose per day, and 2 rats receiving corticosterone developed glycosuria up to a maximum value of over 2 gm. per day. None of the animals given smaller doses of the steroids developed glycosuria.

Blood glucose. Only a small number of determinations of blood glucose were done in these experiments. In confirmation of an earlier study (Ingle, Sheppard, Evans and Kuizenga, 1945) it was shown that the rats tolerated the high carbohydrate diet without the de-

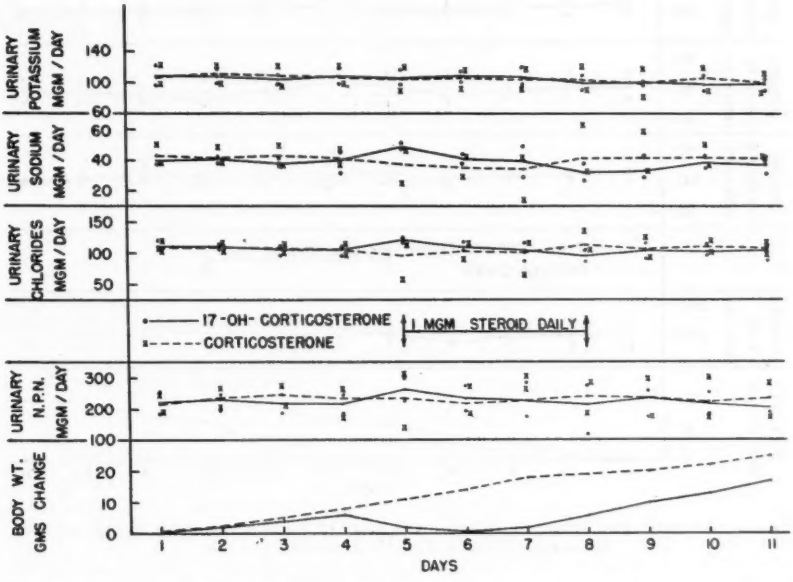

FIG. 3. Averages and range of values for 5 pairs of normal rats at a dosage level of 1 mgm. of adrenal cortical steroids per day.

velopment of hyperglycemia during the control periods and that during the injection of the steroid glucose was excreted only at hyperglycemic levels. Blood glucose was not studied during the administration of the steroids in doses smaller than 5 mgm. per day.

Body weight. The rats each gained weight during the control periods. There was a marked difference in the effect of the two steroids on weight. At a dosage level of 5 mgm. per day, each compound caused a loss of weight, more marked with 17-hydroxycorticosterone. Two mgm. of corticosterone per day inhibited the gain in weight and 17-hydroxycorticosterone caused a definite loss of weight. At dosage levels of 0.5 and 1 mgm. per day, corticosterone did not significantly

inhibit gains in weight. At 1 mgm. per day, 17-hydroxycorticosterone caused a loss of weight, and 0.5 mgm. per day inhibited the gain in weight.

Urinary non-protein-nitrogen. The administration of 17-hydroxycorticosterone caused an increase in the excretion of nitrogen to an extent that was proportional to the dose. The administration of 5 mgm. of corticosterone per day caused an increase in the excretion of nitrogen which was less than the rise caused by an equal amount of

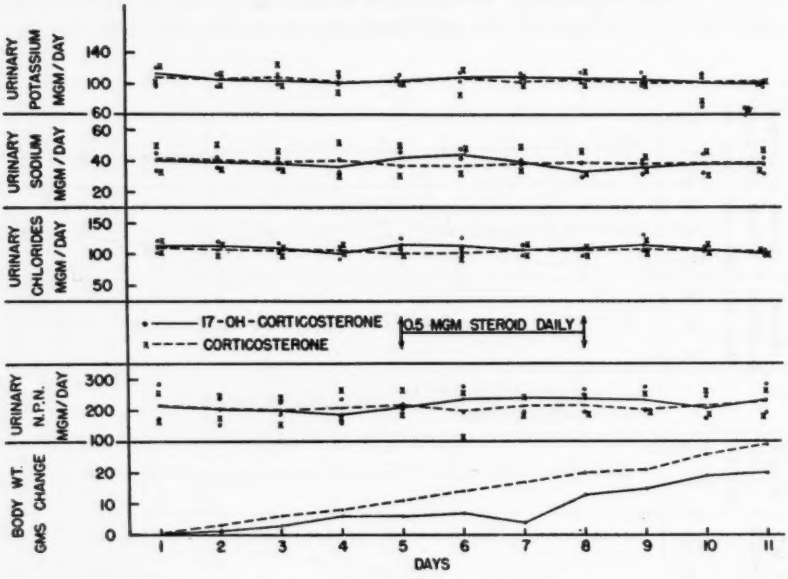

FIG. 4. Averages and range of values for 5 pairs of normal rats at a dosage of leve 0.5 mgm. of adrenal cortical steroids per day.

17-hydroxycorticosterone. The smaller doses of corticosterone failed to cause any significant rise in nitrogen excretion.

Urinary sodium and chloride. In confirmation of an earlier study (Ingle, Sheppard, Evans and Kuizenga, 1945) the injection of 17-hydroxycorticosterone caused a temporary increase in the excretion of both sodium and chloride to an extent that was proportional to the dose. At a dosage level of 5 mgm. daily, corticosterone caused a rise in the excretion of both sodium and chloride, but at dosage levels of 0.5 and 1 mgm. per day there was a slight, possibly insignificant, decrease in the excretion of sodium and chloride.

Urinary potassium. The only significant change in the amount of urinary potassium was a rise in response to the daily injection of 5 mgm. of each compound, more marked in the case of 17-hydroxycorticosterone.

DISCUSSION

These data confirm earlier conclusions (Ingle, 1945) that there are quantitative differences in the biologic properties of corticosterone and 17-hydroxycorticosterone. In respect to effects on weight and the metabolism of protein and carbohydrate, 17-hydroxycorticosterone is the more potent of the two compounds. It is of interest that large doses of corticosterone cause glycosuria and hyperglycemia in the normal force-fed rat, although the changes from normal were less marked than were caused by 17-hydroxycorticosterone.

In these and other experiments (reviewed by Ingle, 1945), moderate doses of corticosterone caused some retention of sodium when administered to normal animals. Large doses (5 mgm. per day) of corticosterone caused a temporary sodium and chloride diuresis just as does 17-hydroxycorticosterone in moderate and large doses. The 17-hydroxy derivative has never been reported to cause sodium retention. In this respect, does it differ qualitatively from corticosterone, or is the apparent difference observed in these experiments a matter of relative potency and dosage? The amplitude of change and the numbers of animals tested were too small to provide a satisfactory answer to this question. Is the sodium diuresis which follows the administration of the C-11, 17-oxygenated adrenal steroids a physiological property of these hormones, or is it a non-specific response to a sudden metabolic upset? This question has been discussed elsewhere (Ingle, 1945; and Ingle, Sheppard, Evans and Kuizenga, 1945) and cannot yet be answered satisfactorily.

SUMMARY

Male rats of approximately 300 gm. initial weight were force-fed a high carbohydrate diet. Glycosuria with hyperglycemia was induced in 4 of 5 rats given 5 mgm. of 17-hydroxycorticosterone per day and in 2 of 5 rats given 5 mgm. of corticosterone per day. At this dosage level, an increase in the excretion of sodium, chloride, potassium and nitrogen was caused by each compound, the greatest response being caused by the 17-oxygenated compound. At smaller doses 17-hydroxycorticosterone, but not corticosterone, caused a temporary increase in sodium excretion, and the latter compound had less effect on weight and nitrogen balance.

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ABSTRACTS OF PAPERS

Presented at Twenty-Eighth Annual Meeting
San Francisco, California, June 28–29, 1946

1. Psychosomatic Aspects of Endocrinology.

Harry B. Friedgood. From the Department of Medicine of the University of Southern California Medical School and the Los Angeles County Hospital, Los Angeles, California.

Carefully conducted observations were made of cases in which emotional conflicts precipitated endocrinopathies such as exophthalmic goiter, adiposogenital dystrophy, amenorrhoea, meno-metrorrhagia, dysmenorrhoea and diabetes mellitus. Other cases were studied in which personality changes and abnormal emotional behavior resulted from hypothalamo-hypophysial tumors and from Cushing's syndrome due to adrenocortical neoplasm. These data indicate that the nervous system influences the functional activity of certain endocrine glands either by their innervation or through neuro-humoral means, and that an abnormal supply of hormones may affect the behavior pattern in certain circumstances.

Correlation of the available anatomical, physiological and clinical data discloses that the hypothalamo-hypophysial area plays an important role in the mechanism by means of which emotional conflicts directly or indirectly disturb the functional activity of certain endocrine organs.

2. Psychic and Endocrine Factors in Obesity.

S. Charles Freed. San Francisco.

Overweight is with rare exception a non-endocrine disturbance. It is a result of a positive caloric balance in which the patient expends less calories in energy than that which is taken in. The actual number of calories required for maintenance of weight may vary widely in different individuals thus accounting for popular misconceptions. The inability to control the intake of food at that level which maintains weight is due to underlying psychic drives. This tendency may be masked for many years or may be present from infancy. Many psychic factors will increase the urge to eat and the inability to control this urge. Other factors include disturbances in the endocrine system, organic diseases and others, but they all have one thing in common in that they increase the psychic or nervous tension of the individual. The most common endocrine disturbances in this regard are the menopause and premenstrual tension, and only occasionally a thyroid deficiency. The treatment of obesity is similar to that of many psychosomatic disturbances The first goal is that of inducing a sense of well-being in the patient by correcting any organic, endocrine or psychic disorder. In addition, benzedrine sulphate is a valuable drug for curbing the appetite and so establishing a high morale so that the patient is encouraged to continue depriving himself of the pleasure of eating. The use of thyroid is only rarely indicated, and often fails besides inducing harm from its toxic effects. Patients should receive psychiatric consultation where mild psychotherapy fails. Proper education of parents and emphasis on the psychological aspects of nutrition for children is advised.

3. Control of Endocrine Obesity.

M. M. Kunde. From the Northwestern University Medical School and the Outpatient Endocrine Clinic of the Cook County Hospital, Chicago.

The investigation of obesity presented in this study is characterized by the fact that all cases herein reported were previously diagnosed as obese due to some endocrine dysfunction; in addition to the adiposity some patients gave evidence of specific gonad, thyroid or other endocrine dysfunction. In the treatment no endocrine product was used as part of the reducing therapy; reduction in weight

was not based on calculated caloric intake but on re-education of the patient's dietary habits.

Physical examination and laboratory tests were made both at the beginning and at the end of the period of weight reduction. In some instances the laboratory findings were checked at the time when the patient had lost 50 pounds in body weight. Laboratory tests, in most cases, included basal metabolism, urinalysis, complete blood count, glucose tolerance, blood cholesterol, calcium and phosphorus, and x-ray of the pituitary. No patients with diabetes or marked hypothyroidism were included in this study. The advice and services of dietitians were not employed. Weight reduction in these patients was handled as any other major medical problem and patients reported directly to the physician at each visit. Charts, graphs and other significant data including weight loss up to one hundred pounds are presented.

4. Three Unusual Non-neoplastic Ovarian Endocrinopathies.

Minnie B. Goldberg, Alice F. Maxwell, and Pearl M. Smith, San Francisco. From the Divisions of Medicine and Pathology, University of California Medical School, and the Hospital for Women and Children, San Francisco.

The three cases with which this paper deals are being reported (1) because they are rare, and (2) because all were diagnosed preoperatively, verified by surgical exploration, and benefited by appropriate therapy.

I. Ovarian Agenesis

An eight-year-old girl was studied for five years because of failure to grow. Ovarian agenesis was suspected and verified by exploratory laparotomy at age thirteen. Early proof of diagnosis was sought in the hope of producing maximum growth before epiphyseal closure occurred. Stilbestrol therapy has been instituted. This is the youngest verified case on record.

II. Ovarian Hyperthecosis

An eighteen-year-old girl with primary amenorrhea, heterosexual hypertrichosis, virilism, acne and impaired carbohydrate tolerance had bilaterally enlarged ovaries. Hyperthecosis of the ovaries was suspected and proved pathologically. Bilateral partial ovarian resection resulted in prompt onset of normal and regularly recurring menses for the past year, and some improvement of the hypertrichosis, acne and carbohydrate tolerance.

III. Precocious Puberty

A six-year-old girl with evident secondary sex characteristics and an adult sized uterus bled per vaginam for eleven days. By exclusion an ovarian tumor was diagnosed despite lack of palpatory evidence. Surgical removal of a unilateral, enlarged, polycystic ovary was followed by regression of all signs of precocious puberty.

5. Bilateral Cryptorchidism in Identical Twins.

S. J. Glass. From the Endocrine Clinic—Cedars of Lebanon Hospital, Los Angeles, California.

The infrequency of reports of congenital sexual anomalies in twins prompts this report. A pair of identical twins presented themselves at age 9 with bilateral cryptorchidism. This was associated with mild stunting of growth and a poor nutritional status. There were no other significant anomalies. Treatment was directed to (1) attempts to induce spontaneous testicular descent with the aid of chorionic gonadotropin,—later supplemented with testosterone proprionate and methyl testosterone in minimal dosage, (2) improvement in nutrition. In two years of observation there was unilateral descent of the contralateral testis in each boy and

descent to the external inguinal ring of the other testis. Considerable acceleration in skeletal and phallic growth accompanied the testicular descent. The nutritional status was likewise improved. Absence from the clinic has prevented further treatment.

6. Carcinoma of the Testis.

Ralph Arthur Reynolds. San Francisco, California.

Zondek, 1930, was the first to report that tumors of the testicle cause increased excretion of the gonadotropic hormone. This discovery has greatly accelerated interest in early diagnosis and treatment of tumors of the testis. The question of pathogenesis remains unchanged. There is still considerable confusion concerning the classification of testicular neoplasms. Correlation between their structure and the hormonal test is resulting in better classification. Chorion epithelioma causes five times the excretion of prolan A as noted in embryonic carcinoma. Persistence of prolan A excretion after excision of the tumor or after irradiation is presumptive evidence of metastasis and therefore of unfavorable prognosis.

A review of a study by Hinman of 85,000 male admissions to San Francisco County and University of California Hospitals showed proved tumors of the testis in 52 or 1:1600. The relative frequency of testicular tumor among malignant tumors of all kinds is about 0.57%. Bilateral involvement, usually in cryptorchidism, is rare. The large majority of patients are between twenty and forty years. Julien, in 1925, collected 139 cases in infants. About 50% of testicular tumors belong to the differentiated type of the carcinomatous group of embryonal tumors. These afford the best prognosis. Primitive types of the carcinomatous group of embryonal tumors are highly malignant and offer a poor prognosis, although highly sensitive to irradiation. Radical surgical treatment of observed metastasis is of no use. Intensive irradiation alone is indicated.

Report of Two Cases

1. Twenty-seven year old male—Orchidectomy performed within three weeks after first observation by the patient. Patient living and well ten years later.

2. Thirty year old male—Reported swelling three and one-half months after first noted by patient. Extensive metastases already present, intensive irradiation instituted. Patient died eighteen months after orchidectomy. Both cases revealed tumors of adeno-carcinoma pattern.

7. Preliminary Report on Protein-Bound Iodine Studies in Thyroid Diseases.

Mayo H. Soley. University of California Hospital, San Francisco.

The determination of the protein-bound iodine in the serum of approximately 400 patients with various types of thyroid disease correlate well with the patients' clinical status and other indirect tests such as the basal metabolic rate, the plasma cholesterol and the oral galactose tolerance test. Sources of error such as previous administration of organic iodine (Priodax and other dyes for gall bladder visualization; Diodrast, Hippuran, Neo-Ipax, Skiodan, Lipiodol and other diagnostic agents) must be recognized.

Follow-up studies in patients with either Graves' disease or hypothyroidism indicate that the return of the clinical status to normal is associated with a similar reversion of the protein-bound iodine to normal levels.

8. Anatomical Evidence of Thyroid Hypofunction in Alloxan Induced Diabetes Mellitus.

Leslie L. Bennett, Alexei A. Koneff and Adrienne P. Applegarth. Institute of Experimental Biology, University of California.

Male rats were rendered diabetic by the intraperitoneal injection of alloxan. There was a reduction in both the relative and absolute thyroid weights in diabetic

animals that was most marked in those with sustained severe diabetes. The mean thyroid weight of animals with diabetes of one month's duration was 16.0—0.8 mgm as compared with 40.3–2.6 mgm for the controls of the same age. Histologically the glands were characterized by small follicles, flattened epithelium, smaller and more darkly stained nuclei, and decreased vascularity. The Golgi apparatus was smaller and less elaborate and appeared as a small cap above the nucleus without the perinuclear extension seen in the normal control. The mitochondria were reduced in number and lost their tendency to be concentrated in the cytoplasm nearest the cell.

9. The Effect of Estrogens on the Thyroid.

F. X. Gassner. Colorado Experiment Station, Colorado State College, Fort Collins.

The thyroids, ovaries and uteri of 104 female rats were examined histologically to determine the effects of continued administration of natural and synthetic estrogens upon these organs, and to obtain information regarding thyroid-ovarian relationship. The animals were kept on a goitrogenic diet, low in iodine, and 3 out of the 6 groups received iodine in the form of KI. Four groups received either 30 gamma estrone or 9.4 gamma stilbestrol daily. The hyperplastic goiter produced in rats on the low-iodine diet was alleviated following administration of iodine. Estrogens given to rats on low-iodine diet reduced the goiter but caused some degeneration of the thyroid parenchyma and lowered iodine storage. Of the two estrogens, diethylstilbestrol was more effective in this respect than was estrone. The storage of iodine in the thyroid glands of rats on low-iodine diet was not interfered with by estrogens when additional iodine was administered daily. This suggests that the effect of iodine on the thyroid is direct and not over the pituitary.

Epidermoid metaplasia occurred in the thyroids of all estrogen-treated rats. It is concluded that metaplasia was caused by the action of estrogens either upon the epithelium of pre-existing follicles because of their cellular duality, or upon embryonal remnants of the resorbed ultimobranchial body. It is not considered likely that an avitaminosis-A was present nor that a deficiency of this vitamin caused the metaplasia observed. The ovaries and uteri of the control rats were essentially normal. The ovaries of rats treated with estrogens showed severe atrophy, follicular atresia, interstitial fibrosis and occasional sub-mesovarial cyst formation. Corpora lutea were generally absent. The uteri of estrogen-treated rats showed squamous metaplasia of the endometrium with desquamation, vacuolar or hydropic degeneration and epithelial tufting. These changes varied in extent from small, focal lesions to total metaplasia of the entire lining of the uterine lumen. The few glands present were also altered.

The significance of certain pathological lesions found is discussed in the light of evidence presented by other workers. It is considered possible that some of the metaplastic areas may constitute a stage in the formation of precancerous foci.

10. Factors Influencing Insulin Production.

Evelyn Anderson, Joseph A. Long, and Virginia Sutton. Institute of Experimental Biology and Division of Medicine, University of California, Berkeley and San Francisco.

With the use of a small perfusion pump circulation was maintained in the isolated rat pancreas (including also the stomach, duodenum and mesentery) for two hours. Evidence of a living organ was demonstrated by its utilization of oxygen and glucose and by the stimulation of insulin production when the glucose in the blood perfusate was raised to 300 mg. per cent. The assay of insulin in the blood perfusate was done on demedullated, alloxan-treated, hypophysectomized rats.

Studies are being made to determine what conditions besides hyperglycemia will stimulate insulin production directly. A purified growth hormone preparation added to the perfusate did not stimulate the pancreas to produce insulin.

11. Biological Assay of Insulin. Objective Determination of the Quantal Response of Mice.

R. E. Thompson (Introduced by F. C. Koch). Pharmacology Section, Armour Laboratories, Chicago.

A simple method is described for the objective determination of the presence or absence of advanced insulin symptoms in mice. The method is utilized in the biological assay of insulin to eliminate personal bias and to reduce the cost of assay by making possible the further use of mice which would ordinarily be discarded. Personnel requirements are also reduced because the close observation of mice ordinarily required is eliminated.

The method consists of placing appropriate insulin treated groups of mice on separate 60° sloped wire mesh screens (24″×36″). A hundred or more mice may be placed on each such screen. The screens must be arranged so that the mice can come off only over a free edge 8 inches or more from any surface. The mice will remain on the screen until advanced insulin symptoms (convulsion, coma, muscular weakness) cause them to lose their foothold. They will then fall from the free edge of the screen into suitable trays after which they may be injected with glucose solution so they will recover for use in subsequent assays. Two hours after injection the mice remaining on the screens are counted. Difference from the original number gives the number of positive reactions. The customary treatment of these values gives the estimated potency of the sample insulin in terms of a standard.

Over 100 assays (200 mice each) have been completed by this procedure at room temperature. The procedure is shown to be equivalent in precision to the usual procedure of detecting positive reactions by observation of mice in ordinary temperature controlled compartments.

This "sloped screen" procedure is adaptable to either room temperature or elevated temperature operation.

It is pointed out that this procedure for objectively determining a physiological "end-point" may be adaptable to other problems such as the bioassay of adrenocorticotropin and adrenocortical extract by anti-insulin activity and the quantitative study of hypnotics and anesthetics.

12. Insulin Resistance: A Case Study.

Thomas H. McGavack, Solomon D. Klotz, Mildred Vogel and James F. Hart. N. Y. Medical College, Metropolitan Hospital Research Unit.

A 64 year old man, whose diabetes began 7 years ago, demanded at that time as much as 920 units intramuscularly and 400 units intravenously daily to overcome glycosuria and ketonuria. Later he was able to do without insulin for 3 years. A second and a third period of "insulin insensitivity" have been observed, each associated with severe local reactions to insulin injections. As a result of studies during this third period, allergic antibodies to insulin were demonstrated repeatedly by the Kustner-Prausnitz method for passive transfer of sensitivity. Such reactions disappeared in from 10 to 12 weeks after the discontinuance of insulin and reappeared within from 10 days to 2 weeks upon resumption of the same. 'Benadryl' protected the patient from the development of insulin resistance due to these antibodies but did not cause their disappearance.

Effort was made to determine the presence of other substances in the blood and tissues of this patient which possessed a direct anti-insulinic action capable of protecting mice from ordinarily fatal doses of insulin. All attempts to demonstrate such a substance have failed, even when the patient was receiving 400 units of insulin daily and showing ketonuria and glycosuria of high degree.

It is concluded that insulin may act as an allergen; the development of antibodies may be so great that severe resistance to the action of insulin results. The data are discussed in relation to previous concepts of insulin resistance as revealed in the literature.

13. The Effects of Growth Hormone and Adrenocorticotropic Hormone on the Urinary Glucose and Nitrogen Excretion of Diabetic Rats.

Leslie L. Bennett and Choh Hao Li. Institute of Experimental Biology, University of California.

Rats with persistent diabetes mellitus induced by alloxan were maintanied on a constant food intake in metabolism cages. Urinary nitrogen and glucose were determined daily. The effects of three mgm per day of growth hormone and adrenocorticotropic hormone were studied. In each of thirteen experiments adrenocorticotropic hormone increased the degree of glycosuria and nitrogen excretion both with and without exogenous insulin administration. The increase in urinary glucose was greater than could be accounted for by gluconeogenesis from the extra protein catabolized under the influence of adrenocorticotropic hormone as measured by the rise in urinary nitrogen.

In seven of eight experiments without exogenous insulin, and in each of five experiments with exogenous insulin, growth hormone promoted protein storage as indicated by a decrease in urinary nitrogen excretion. In only five of the thirteen experiments with growth hormone was there a significant increase in glycosuria.

There is, therefore, in rats with alloxan induced diabetes a clear cut separation between the metabolic effects of growth hormone and adrenocorticotropic hormone. Only the adrenocorticotropic hormone produced a typical exacerbation of the diabetes with enhancement of both glycosuria and nitrogen excretion.

14. A Comparison of the Acute Effects of Corticosterone and 17-Hydroxy-Corticosterone on Body Weight and the Urinary Excretion of Sodium, Chloride, Potassium, Nitrogen and Glucose.

Dwight J. Ingle, Ruth Sheppard and Marvin H. Kuizenga. The Research Laboratories, The Upjohn Company, Kalamazoo.

Normal male rats were force-fed a high carbohydrate diet. Five rats (total of 40) were tested at each dosage level of each of the two steroids. The administration of 5 mgm per day for five days of either corticosterone or 17-hydroxycorticosterone caused the excretion of glucose by some of the rats and a loss of weight and an increased excretion of sodium, chloride, potassium and nitrogen by all of them. The responses to the 17-oxygenated compound were greater than the responses to corticosterone. At dosage levels of 0.5, 1, and 2 mgm. per day for three days no glucose was excreted. At each dosage level 17-hydroxycorticosterone caused a loss of weight and an increased excretion of electrolytes and nitrogen to an extent which was proportional to the dose. At a dosage level of 2 mgm. per day corticosterone caused some inhibition of weight gain but did not have a consistent effect on the excretion of electrolytes and nitrogen. At dosage levels of 0.5 and 1 mg. per day, corticosterone caused some retention of sodium and of chloride but there was little effect on the excretion of potassium and nitrogen.

15. The Isolation of a New Active Steroid from the Adrenal Cortex.

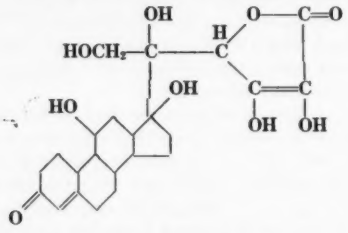
Bertrand E. Lowenstein* and R. L. Zwemer.† From the Department of Anatomy, College of Physicians and Surgeons, Columbia University and the Department of Pharmacotherapy, Harvard University Medical School.

An attempt has been made to isolate an active compound from aqueous extracts of adrenal glands. Fresh adrenal glands were extracted with $CHCl_3$ in the presence of enough anhydrous Na_2SO_4 to remove all the water present. The extract was purified of inactive steroids by repeated distribution between water and benzene, and the active material finally adsorbed on a precipitate of colloidal $FeSO_4$ from an aqueous-alcoholic solution.

* Present address: Research Division, Cleveland Clinic.

† Present address: Assoc. Chief, Division of Cultural Relations, State Department, Washington, D. C.

Purification of the active material thus adsorbed has resulted in the separation by fractional crystallization of dehydrocorticosterone, and of two hitherto unisolated compounds whose combined activity accounts for about 80% of the activity of the original aqueous extract. One of these compounds has been further characterized and is a ketonic steroid with empirical formula $C_{25}H_{34-36}O_9$. On mild hydrolysis in the absence of air ascorbic acid has been isolated and identified chemically and biologically. Oxidation with periodic acid or chromic acid yields adrenosterone. The following structural formula is tentatively ascribed to it:



Tests of the activity of this compound indicate that it is as active or more active than desoxycorticosterone in life-maintenance, somewhat less active in its effect on sodium retention, and about equal to dehydrocorticosterone as far as carbohydrate activity is concerned.

16. The Quantitation of Axillary Hair Growth as an Index of Endocrine Function.

Laurance W. Kinsell, Edw. C. Reifenstein Jr., Dorothy Bryant, and Fuller Albright. Massachusetts General Hospital, Boston.

The growth of axillary hair has been quantitated in normal individuals, and in patients with various endocrinopathies. The work was undertaken on the hypothesis that there would be a direct correlation between the growth of genital hair and the excretion of 17 keto-steroids. This has been found to be true only in certain categories. There was however, found to be a direct correlation between the growth of axillary hair, and the local and systemic administration of androgen. Considerable data on axillary hair growth in idiopathic and endocrine hirsutism is presented, and correlated with keto-steroid out-put, and other objective laboratory data.

On the basis of the data so far obtained, it is felt that the quantitation of genital (axillary) hair represents a simple diagnostic measure of considerable usefulness in the evaluation of endocrinopathies.

17. The Assessment of Somatic Androgyny.

Nancy Bayley, and Leona M. Bayer. From the Institute of Child Welfare of the University of California, and the Stanford University School of Medicine.

A rating scale has been devised for assesing somatic androgyny in almost mature boys and girls. Standards are presented by body photographs; descriptions; a rating chart for constructing individual profiles. Subjects are rated according to degree and direction of sexual differentiation. Agreement between independent raters shows the scale to be reliable.

Scores fall into a bi-modal continuum with small amounts of overlapping of sexes. Cases with intermediate scores may be either undifferentiated (asexual), or show characteristics of the opposite sex (bisexual).

Androgyny appears to be truly a "mosaic," as Draper so aptly labeled it. Valid sex differences, having little or no overlap between masculine and feminine scores

are almost entirely independent of each other within a sex. Many individuals may deviate in a few characteristics; few will deviate in many.

With standards for assessing somatic androgyny it should be possible to determine its relation to personality structure. Significant personality differences may well be found between those with normal builds; with relatively immature builds; with build characteristics of the opposite sex. Perhaps androgyny scores will also prove useful in relating sex variations in physique to other aspects of body build; to physical fitness; to hormone assays.

18. Sexual Behavior of the Bovine Male Used in Artificial Insemination.

G. H. Hart, W. M. Regan and S. W. Mead (Introduced by H. H. Cole). Division of Animal Husbandry, University of California, Davis.

Artificial insemination is now widely used in many countries of the world on several species of domestic animals, particularly dairy cattle and sheep. The techniques have been extensively studied and are reasonably well standardized.

One of the serious limiting factors is the behavior of the male in semen collection. Sexual libido, quality and quantity of the ejaculate and longevity of sperm activity are all factors subject to great variation within short periods of time in the same animal. Each animal manifests distinctly individualistic characteristics. Heredity and environment are both involved and need to be differentiated. Factors for sterility are evidently involved with good blood lines for production. Proven sires for transmitting ability with high fertility are to be desired in order to eliminate tendency toward male sterility. The paper sets forth the problem with some data without answering the questions involved.

19. Endocrine Feature and Treatment of Chronic Cystic Mastitis and Their Relation to Infertility.

Joseph H. Morton. From the Department of Medicine, N. Y. Medical College, Flower and Fifth Avenue Hospitals—and the Endocrine Service of Dr. T. H. McGavack at Metropolitan Hospital.

Mammary growth and development have been shown to be dependent largely upon the ovarian hormones. In general it has been accepted that estrogens mediate the development of the mammary ducts and progesterone that of the lobular alveoli. The role played by the anterior pituitary is still not entirely clear.

The breast, like the endometrium, cyclically shows a proliferative phase induced by estrogens, and a secretory phase stimulated by progesterone. Excessive estrogenic activity, relative or absolute (with an associated diminution in progesterone), may result in abnormal changes known as chronic cystic mastitis.

The author stresses the frequent coincidence of chronic cystic mastitis and infertility in a group of women showing no gross pelvic pathology. In these patients special tests such as the basal metabolic rate, postcoital examination, tubal insufflation test and the seminal fluid were normal. The endometrial biopsies, however, showed evidence of hyperestrinism. The author concludes that the endometrial and mammary abnormalities are both manifestations of disturbed estrogen-progesterone balance.

Several methods of hormonal therapy, and also the place of irradiation and surgery in the management of chronic cystic mastitis are discussed and evaluated. Chorionic gonadotropin was found to be the most effective method of therapy.

20. Artificial Reproduction of the Cyclic Changes in Cervical Mucus in Human Castrates.

A. R. Abarbanel. Departments of Obstetrics and Gynecology, College of Medical Evangelists Los Angeles.

By means of estrogen (estradiol, diethylstillbestrol, hexestrol, estrone) the castrate's cervix was stimulated to secrete a clear glairy mucus which human sperm were able to penetrate and migrate through.

When progesterone or ethinyl testosterone (anhydro-oxy-progesterone) was given, the mucus became more viscid, decreased in volume, while sperm either were unable to penetrate or if they did, they soon bogged down. The relationship of these findings to the cervical factor in infertility is discussed.

21. Further Studies on the Control of Menorrhagia.

Robert B. Greenblatt. University of Georgia School of Medicine, Department of Endocrinology, Augusta, Georgia.

1. The examination of the endometrium soon after arrest of menorrhagia by estrogenic therapy usually reveals a hyperplastic or a proliferative endometrium.

2. When moderate doses of androgenic therapy are employed and bleeding is arrested, the endometrium is maintained apparently in the same status as prior to therapy.

3. When progesterone therapy is used and bleeding is arrested "according to plan," i.e. about 7–10 days after cessation of therapy, shedding of the endometrium occurs, comparable to that which occurs with normal physiologic menstruation. However, the loss of blood during the 7–10 day period may be excessive.

4. The administration of 25 mg. of testosterone propionate and 10 mg. of progesterone daily over a three day period results in almost immediate cessation of bleeding which, after an interval of 4 or 5 days, is followed by a short bout of withdrawal bleeding.

5. In the study, over a period of many years, of functional uterine bleeding, this method of therapy has thus far proved the most promising and ideal in the management of menorrhagia.

22. Factors Concerned in the Maintenance of Function of the Corpus Luteum.

Warren O. Nelson. University of Iowa, College of Medicine, Iowa City.

Estrogenic hormone appears to be a factor in the maintenance of functional activity in the corpora lutea of some species although the nature of this relationship may not be constant in the various forms. Administration of estrogen to adult female rats resulted in the maintenance of luteal function for 15 to 20 days, but did so only in the presence of the hypophysis. Removal of the hypophysis at any time during the course of treatment, i.e., during the course of pseudopregnancy, was followed by a return of the vaginal smear to an estrous state within a few days, as was true if the ovaries were removed.

Animals in which the hypophyses were removed during the course of pseudopregnancy, estrogen treatment being continued, returned to estrus when FSH, LH, chorionic gonadotrophin or pregnant mare serum were given. However, the administration of lactogenic hormone in such animals maintained the pseudopregnant state for as long as 20 days post-hypophysectomy. Suspension of treatment at any time in such animals was followed by the occurrence of vaginal estrus within three days. Animals sacrificed during the period of pseudopregnancy showed large corpora lutea, well-developed mammary glands, and mucified vaginae. Suitable stimulation of the uteri of pseudopregnant animals was followed by the development of deciduomata.

The effectiveness of preparations of lactogenic hormone in maintaining corpora lutea is attributed to the presence of the luteotrophic factor. The latter appears to be identical with the lactogenic hormone since the preparations show parallel activity of the two factors.

23. Induction of Ovulation in the Diestrous Mouse by Gonadotropins.

Francis J. Saunders. From the Research Laboratories of G. D. Searle & Co., Chicago.

Various gonadotropins were compared as to their ability to produce uterine weight increases in immature rats and ovulation in adult diestrous mice. After

standardizing various preparations on the basis of their effectiveness in increasing uterine weight in immature rats, it was found that about 9 R.U. of pregnancy urine extract were required to cause ovulation in 50% of the mice, or about 4 or 5 R.U. of pregnant mare's serum. Extracts of pituitaries prepared by the same method, but from glands from different species, were studied as were also various types of extracts from sheep pituitary glands. It was found that 0.1 to 1.5 R.U. of the various pituitary extracts evoked ovulation in 50% of the mice.

24. Diagnosis of Hydatidiform Mole by Gonadotrophic Hormone Assay Using the South African Frog.

Abner I. Weisman and Christopher W. Coates (Ft. Thompson, S. Dak.) & (New York).

A simple and rapid method of assaying large amounts of chorionic gonadotrophic hormone excreted in the urine of patients with a suspected chorionic tumor (hydatidiform mole) is presented. By means of injecting varying dilutions of the suspected urine into pairs of the South African Frog (*Xenopus laevis*) a six-hour response is obtained. The reaction is an extrusion of ova following injection of the urine containing the hormone. The method used is to dilute the urine 1:10 and 1:100 and then inject one cubic centimeter of each dilution into each of two frogs. If the 1:100 dilution causes extrusion of ova in the frogs, the amount of gonadotrophic hormone can be assumed to be present in at least 100,000 frog units per liter. No other condition, but chorionic tumors of the hydatid variety, will evoke such a strong hormone response. This test may then be assumed as a specific test for hydatidiform mole. Six cases are reported to cite the pre-operative diagnosis by this method.

25. The Influence of Various Hormones on the Resistance of Swiss Mice to Adapted Poliomyelitis Virus.

John A. Anderson and Vernon Bolin. Dept. of Pediatrics, University of Utah, School of Medicine, Salt Lake City.

The nature of autarceologic immunity in man and animals to poliomyelitis virus is unknown. Spontaneous mouse poliomyelitis virus (Theilers) may infect 100% of Swiss mice yet only one in 2,000 animals succumb to paralysis. It is generally believed that a sub-clinical form of poliomyelitis affects the majority of a human urban community with development of only occasional paralytic conditions. Since estrogen treatment of animals reduces mortality from intranasal doses of an adapted poliomyelitis virus it has been suggested that physiological disturbances in metabolism of hormones may play a role in the autarceologic immunity of man.

Swiss mice capable of being orally infected with the adapted poliomyelitis virus (Jungeblut-Dalldorf MM strain) were treated with desoxycorticosterone acetate, progesterone, t-propionate and stilbesterol and the mortality rate to orally administered virus determined. Control and experimental animals were fed a dose required to kill 68% of the animals.

It was found that protection against the orally administered virus was provided in the following declining order—progesterone (complete protection), stilbesterol (mortality reduced from 68% to 2.5%), t-propionate (68% to 20%); desoxycorticosterone acetate failed to modify mortality.

It is possible that these substances modify mucous surfaces, preventing entry of the virus into the body, or stimulate effective immune responses.

26. The Validity of Endocrine Research on the Domestic Norway Rat.

Curt P. Richter. Phipps Clinic, Johns Hopkins Hospital, Baltimore.

Evidence gathered during the past few years indicates that the domestic Norway rat commonly used for all types of research in endocrinology and allied fields may

be nothing more than an artificial creation of the laboratory experimenter produced by a long period of selective breeding under unnatural conditions.

This statement is based on comparisons made between domestic Norways and many thousand wild Norway rats recently trapped from the streets and alleys of a large city. These comparisons have revealed the existence of great differences in the anatomy, physiology, and responses to drugs in these two forms.

One of these differences which is of special interest to endocrinologists concerns the anatomy and function of the adrenal glands. In the domestic Norway the gland is about one-third as large as it is in the wild rat owing to a great atrophy of the cortex. (The medullas are about the same size.)

Adrenalectomy in the wild rat produces a much more pronounced state of shock. Even when they receive an amount of salt which suffices for domestic rats, these wild rats do not survive. They refuse to eat or drink, and die within two weeks. Clearly the adrenal cortex plays a much more important part in wild rats with their marked tension, fear, and anxiety, than it does in the tame, placid, domestic rats. Experiments are in progress to determine which of the various cortical extracts and in what amounts will be required to keep these rats alive. Clearly maintenance after adrenalectomy is a much more difficult problem than in the domestic rat.

A few of the other differences are that thiourea which has such a pronounced effect on the thyroid gland kills the domestic rat in doses as low as 3–5 mg. per kilogram body weight while 1–2 grams per kilogram body weight are required to kill a wild Norway rat.* Audiogenic stimulation which has no effect on the wild Norway throws the domestic Norway into violent and usually fatal fits and convulsions.

Thus it is possible that much of the research done on the domestic rat will have to be repeated and checked on the more normal wild Norway.

Simple methods for trapping, handling, and housing wild Norway rats will be described.

27. Discussion of a Simple, Rapid and Practical Means of Determining Urinary Gonadotrophins in Patients.

Edwin Jungck,† William Maddock, and Carl G. Heller. Departments of Physiology and Medicine, University of Oregon Medical School, Portland.

Simplicity, rapidity and efficiency are three prime criteria by which any method of assaying urinary gonadotrophins from patients must be judged, if such a method is to be widely adopted in clinical laboratories. Concentrating gonadotrophins from urine by filtering them through collodion membranes using pressure, dissolving the membranes, and then dissolving the active precipitates (as suggested by Gorbman) satisfies the requirements of simplicity and rapidity. To test the efficiency and practicability of this procedure, it was compared with recovery of gonadotrophins using the alcohol precipitation—dialysis—alcohol reprecipitation method.

Urinary gonadotrophin titers of normal males and females, and of patients having a wide variety of syndromes causing both high and low titers were compared. The results indicate that the recovery is less complete using the membrane technic; yet it is sufficiently great and simple to provide an excellent laboratory aid to clinical diagnosis of endocrine disorders.

Details of the method along with gonadotrophic hormone analyses of interesting cases will be presented.

* Dieke, S. H. and C. P. Richter: Acute Toxicity of Thiourea to Rats in Relation to Age, Diet, Strain and Species Variation. Journal of Pharmacology and Experimental Therapeutics, *83:* 195, No. 3, March, 1945.

† Schering Research Fellow in Endocrinology.

28. GONADOTROPHINS IN NERVOUS EXHAUSTION DUE TO OVARIAN HYPOFUNCTION. L. F. Hawkinson. Oakland.

As in the menopausal syndrome, ovarian hypofunction previous to the climacteric often produces distressing symptoms. Equine gonadotrophin, used chiefly in the treatment of menstrual disturbances and sterility was employed in the present series of 460 cases. These patients complained of unpleasant symptoms which included nervousness, fatigability, headache and depression. The ovarian deficiency was usually temporary, nearly one-third followed pregnancy. Vaginal spreads were an aid in diagnosis, as well as in evaluation of treatment. When the syndrome was severe and in those with undesired sterility, one-two cyclic gonadotrophin therapy was used. Although the accompanying menstrual disorder was often corrected when symptoms were relieved, subjective symptoms tended to respond more readily. Subjective symptoms were relieved in 52.6%, and improvement was noted in 32.1%.

29. EQUINE GONADOTROPIN: NINE YEARS OF CLINICAL EXPERIENCE. George Joyce Hall. Sacramento.

Obviously different from the human gonadotropin factor of the anterior portion of the pituitary gland, equine gonadotropin seems at times to have stimulating clinical effects on hypofunctioning human ovaries.

Over twelve hundred patients have received P.M.S. (pregnant mare's serum) for the control of menstrual disturbances and sterility due to hypo-ovarian function during the past nine years.

We insist on the proper "preparation" of the patient before administration of the serum is justified. Control of the "symptoms" by substitution therapy, and obtaining a relatively normal phase of vaginal cornification on the 18th to 23rd day of the ovarian cycle is considered necessary before there is any reason to expect the P.M.S. to cause any improvement of ovarian function. Adequate clinical tests have been made of all patients before and throughout the therapy.

Statistical tables showing results of treatment will be presented, and an effort made to determine the percentage of those whose ovarian function seems normal, but who do not remain stabilized long enough without therapy to be properly classified as "cured."

Results obtained not only justify continued use of P.M.S. for the treatment of hypo-ovarian functional conditions, but seem to indicate more satisfactory results than are obtainable with any other gonadotropin therapy.

30. THE SYNERGISTIC ACTION OF PROGESTERONE WITH TESTOSTERONE PROPIONATE ON THE REPRODUCTIVE TRACT OF THE CASTRATE FEMALE OPOSSUM. Charles F. Morgan. Department of Pharmacology and Materia Medica, Georgetown University, School of Medicine, Washington, D. C.

Progesterone administered alone in 1 mg and 5 mg daily doses for twelve days to the castrate female resulted in a significant decrease in the weight (56%) and size of the reproductive tract as compared with the untreated castrate. The greatest decrease in size and weight was in the uterus.

Testosterone propionate administered alone for twelve days in 1 mg daily doses did not significantly alter the weight or size of the tract but histological studies showed that the tissues were mildly stimulated. T-propionate given in 5 mg daily doses caused the weight and size to increase over the untreated castrate.

Progesterone, 1 mg/day, and t-propionate, 1 mg/day, given simultaneously for twelve days resulted in a synergistic action which increased the weights of the Müllerian duct derivatives of the reproductive tract to about 4 times those of the untreated castrate; about 8 times those of the castrate treated with the same dosage of progesterone alone; and about 4 times those which received the same dosage of t-propionate alone. The derivatives of the urogenital sinus were also affected by a combination of the two hormones, but to a lesser extent than were

Müllerian duct derivatives. The synergism was also present when the daily dosage of t-propionate was increased to 5 mg in combination with 1 mg of progesterone.

Under the conditions of this experiment, (1) progesterone administered alone has no stimulating effect, but appears to cause regressive changes in the reproductive tract; (2) androgens administered alone show a stimulating effect; (3) a synergism between progesterone and t-propionate is manifested when the two hormones are administered simultaneously, and (4) progesterone apparently enhances the action of the androgen.

31. The Source of the Excess Creatine Following Administration of Methyltestosterone.

Leo T. Samuels, Dorothy M. Sellers, Carley J. McCaulay. Department of Biological Chemistry, University of Utah, Salt Lake City.

Since creatinemia and creatinuria do not follow administration of methyltestosterone in other animals, experiments were carried out on human beings with diseases of certain organs that might be involved in creatine metabolism. The following groups were studied: normal subjects, patients were severe liver damage, patients with nephrosis, and patients with severe nephritis. Blood and urine samples were collected before administration of methyltestosterone, after ten days of medication and at the end of twenty days' treatment with the drug. In the normal individual the plasma creatine was generally elevated at the end of the first ten days, but in adult males creatinuria did not always appear because of the low original creatinemia. Creatinemia appeared earlier in women and children, but, in all cases the creatine threshold lay between 0.7 and 1 mg/100 cc plasma. Creatinemia and creatinuria were produced in the cases suffering with hepatic damage, even when in a terminal condition. Creatinemia also appeared in the group suffering with nephrosis. The only cases where no large increase in plasma creatine occurred were those suffering with severe nephritis. Apparently the kidney is a primary factor in the excess creatine found after administration of methyltestosterone.

32. Experimental Use of Testosterone in Premature Infants.

E. Kost Shelton. Los Angeles.

Because of the nitrogen storage factor and other physiologic properties attributed to testosterone, preparations of this substance were administered to premature infants in the hope of stimulating the vital processes measured by survival. The results were encouraging.

33. Craniopharyngioma with Infantilism: Case Report with Clinical and Pathological Studies.

Thomas H. McGavack, Raymond Harris, Andrea Saccone and Harry Goldberg. New York Medical College, Metropolitan Research Unit.

Death of a man at age 54, with first symptoms at age of 15, was preceded by five years continuous observation during which prolonged treatments included methyl testosterone, t-propionate, desoxycorticosterone acetate, alpha-estradiol dipropionate, desiccated thyroid and pituitary preparations; diets of known sodium, carbohydrate, protein and fat were used.

Post-mortem revealed a lack of normal anterior pituitary and the presence of a calcified craniophraryngioma $4 \times 4 \times 3$ cm. Organ weights in grams were thyroid and parathyroids 9.7; right testicle, 2.8; left testicle plus testosterone implant, 4.4; adrenals—right 3.6, left 3.3; prostate, 10.2; pancreas, 49.1; thymus unweighable.

Subjective improvements always followed testosterone therapy (25 mgs or more 3 times weekly) or desiccated thyroid (15–200 mgs daily) or a combination of these. Flat intra-venous glucose tolerance curves of untreated periods became normal under testosterone therapy but were not altered by other treatments; high

cholesterol was reduced to normal by thyroid but unmodified by other medicants. Blood potassium was low,sodium high, with treatments of estradiol and desoxycorticosterone acetate. Fluid intake and urine output rose from 1.0 liter to 3.5 liters per day with desoxycorticosterone, 15 mgs daily, and a diet containing 2.6 gms sodium. Urinary creatine was decreased by t-propionate, increased by methyl testosterone, unchanged by other agents. Low insulin clearances were elevated by DCA and less by t-propionate. Capillary permeability was increased from minus 22% to plus 33% by DCA and less by estradiol; lowest values occurred with thyroid. Kepler-Robinson water tests similar to those in Addison's disease were not altered by treatment. Pituitary extract were ineffective in this patient.

34. Continuous Growth of Normal Rats Receiving Pure Growth Hormone.
Herbert M. Evans, Miriam E. Simpson, and Choh Hao Li. Institute of Experimental Biology, University of California.

Normal, adult, plateaued, female rats (211 days old) have been injected 6 days weekly for 436 days with anterior hypophyseal growth hormone. The initial dose of 0.4 mg was gradually increased to 2 mg. Growth continued during the whole period, the experiment being terminated due to the advanced age of the animals. The greatest weight attained was 662 gm. The range of final weights in the experimental and control groups did not overlap; the smallest experimental rat weighed 410 g. the largest control 353 g. The average gain of 8 experimental rats was 293 g, of 5 controls 57 g. The average body length at autopsy of the experimental rats was 45.5 cm, of the controls 40.9 cm. Liver, kidneys, heart, stomach and intestine increased in weight in proportion to body weight. The thymus was not hypertrophied as occurs upon acute administration of growth hormone. The other endocrine organs were not increased in proportion to body weight, as might be anticipated from the absence of the specific hormonal stimulants in the growth hormone injected.

36. Observations on the Use of the Serum Phosphorus Level as an Index of Pituitary Growth Hormone Activity; The Effect of Estrogen Therapy in Acromegaly.
Edward C. Reifenstein, Jr., Laurence W. Kinsell,; and Fuller Albright. From the Department of Medicine of the Harvard Medical School and from the Medical Service of the Massachusetts General Hospital, Boston.

Three observations are presented in this paper: (1) The serum "inorganic" phosphorus level is elevated without a corresponding fall in the serum calcium level in most acromegalic patients; the same is true in growing children. (2) The high serum phosphorus level and many of the symptoms of acromegaly respond to estrogen therapy. Since it is known from the literature that the administration of estrogen alone inhibits growth in animals and that the simultaneous administration of growth hormone with estrogen prevents this inhibition, it is suggested that (other factors being equal) a high serum phosphorus level may be an index of pituitary growth hormone activity. (3) The negative calcium balance (a common finding in acromegaly) responds to estrogen therapy as does the calcium balance of post-menopausal osteoporosis. The evidence for the above observations and a discussion of certain implications, both academic and clinical, is given in this presentation.

36. Factors Influencing the Production of Cardio-Vascular Diseases by Anterior Pituitary and Corticoid Hormones.
Hans Selye. From The Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada.

Both desoxycorticosterone acetate (DCA) and lyophilized anterior pituitary tissue (LAP) produce malignant hypertension, nephrosclerosis, periarteritis nodosa

of the cardiac vessels and myocarditis (with nodules resembling Aschoff's bodies) in the rat.

Unilateral nephrectomy and a high NaCl or a high protein intake sensitize the rat to these toxic effects of both DCA and LAP; "acidifying salts" (e.g. NH_4Cl or $CaCl_2$) and low protein diets have an opposite effect.

Low protein diets also inhibit, and high protein diets enhance the ability of LAP to cause adrenal cortical hypertrophy.

In the adrenalectomized animal, none of the above-mentioned LAP overdosage manifestations are evident; hence, LAP probably acts because of its adrenotropic hormone content which causes the adrenal cortex to elaborate DCA-like corticoids.

In the case of DCA overdosage, the above-mentioned changes are accompanied by hypopotassemia, hypochloremia and periarteritis nodosa of the mesenteric vessels, while LAP treatment rarely produces these latter changes.

The cause of the slight qualitative differences between the actions of DCA and LAP have not as yet been elucidated. It is possible that the anterior pituitary produces substances which inhibit some of the actions of DCA, or that the natural (endogenous) corticoids liberated by the adrenal, under the influence of LAP, are qualitatively slightly different from DCA.

In rats sensitized by NaCl and unilateral nephrectomy, various types of nonspecific damage (e.g. cold) produce lesions similar to those elicited by LAP or DCA. The theory is advanced that the spontaneous diseases of human pathology, which are imitated by LAP or DCA overdosage, are due to a defensive increase in the production of corticotropic and corticoid hormones. The above-mentioned cardio-vascular lesions could then be regarded as by-products of this adaptive defence reaction; that is, as "Diseases of Adaption." (Subsidized by a grant of the Commonwealth Fund).

37. THE SUBSTANCE IN LATE PREGNANCY MARE SERUM CAUSING OVARIAN INHIBITION.

H. H. Cole. Division of Animal Husbandry, University of California, Davis.

In earlier studies, we (Cole and Hart, 1930) showed that, whereas the serum of mares in early stages of pregnancy contained large amounts of a gonadotrophin, in late pregnancy the serum inhibited ovarian activity in the immature rat. Furthermore, the ovaries of the mare in late pregnancy regress to a remarkable degree and are devoid of both corpora lutea and large follicles.

Because of the large amounts of gonadotrophin present in early pregnancy, the possibility existed that an antigonadotrophin was formed. Tests for antigonadotrophin were negative, however—a further substantiation of the concept enunciated by Leathem and Rakoff that gonadotrophins of homologous source are not antigenic.

Tests for lactogenic hormone, another substance producing ovarian inhibition, were likewise negative.

The evidence at hand indicates that the inhibition is due to estrogens present in the blood in low concentration. The inhibition produced by late pregnancy serum has been compared to that obtained with estradiol benzoate (Progynon B). In both instances, inhibition occurs if a dose is given equivalent to about one-fourth R.U. daily. Estrogens in the urine of pregnant mares produce a similar inhibition at comparable dosage levels.

The role which estrogens play in regulating gonadotrophic secretion will be discussed.

38. The Problem of Antigonadotropins in Clinical Therapy.

J. H. Leathem and A. E. Rakoff. From the Department of Zoology, Rutgers University, New Brunswick, and the Departments of Obstetrics and Gynecology and the Endocrine Laboratory, Jefferson Medical College and Hospital, Philadelphia.

Adequate clinical evidence is available to show that antagonists against equine gonadotropin and synapoidin (sheep pituitary and chorionic gonadotropin) develop in the serum during therapy. Antihormones persist for at least three months and with synapoidin the titres could be correlated with the response to therapy in cases of functional bleeding.

To establish a rationale for prolonged therapy, hormone specificity of antihormones was studied. Serum from patients treated for two to five months with synapoidin failed to reveal antagonists to synapoidin, equine gonadotropin, equine pituitary, chorionic gonadotropin or human pituitary with but one exception in which chorionic gonadotropin was inhibited. Patients who developed antihormones against synapoidin revealed that the antiserum would not antagonize the action of human pituitary but did inhibit equine gonadotropin and chorionic gonadotropin. The non-specific nature of the antiserum is indicated.

Two patients developed antihormones to equine gonadotropin but the serum did not antagonize the action of human pituitary or synapoidin. The antiserum formed against equine gonadotropin appears to be hormone specific.

Synapoidin and equine gonadotropin were administered to one patient at different times and the serum when tested was capable of inhibiting equine gonadotropin and chorionic gonadotropin but did not influence synapoidin.

39. End Results of Treatment of Pituitary Dwarfs with Chorionic Gonadotropin and Sex Hormones.

W. O. Thompson, N. J. Heckel, and P. K. Thompson, Chicago.

Observations on the treatment of pituitary dwarfs during the last sixteen years warrant the following conclusions:

The only way in which growth can be accelerated in male pituitary dwarfs is by the administration of chorionic gonadotropin or male sex hormone. These materials insure that the growth component associated with puberty takes place and are therefore of value only if the gonadotropic-producing mechanism of the pituitary is deficient. They are in no sense a substitute for the thyroid hormone or for the growth factor of the pituitary. They influence growth by inducing puberty and causing the acceleration of growth and skeletal molding associated with puberty. The maximum acceleration of growth associated with the administration of these materials in the male pituitary dwarf is about 10 inches and the optimum time for starting the administration appears to be the eleventh or twelfth year.

In the female pituitary dwarf the administration of female sex hormone causes some acceleration of growth, although not as much as the male sex hormone in the male pituitary dwarf.

The thyroid hormone and various commercial preparations of the growth factor of the pituitary are not effective in stimulating growth in pituitary dwarfs.

Pituitary dwarfs treated with chorionic gonadotropin and the sex hormones remain short because they show only that increment of growth associated with puberty and there is no way at present of making up for the deficiency of pituitary growth factor.

40. Advantages of Modified Protamine Zinc Insulin in the Regulation of Diabetes.

Cyril M. MacBryde. Los Angeles.

A modification of protamine zinc insulin containing 0.5 mg. of protamine per 100 units (instead of the standard 1.2 mg.) has 25 per cent of the insulin content

in soluble, quickly-absorbed form, while 75 per cent is in the precipitate and is slowly absorbed over a period exceeding 24 hours. Maintenance therapy with this MPZ insulin has proved very satisfactory direct comparative studies having shown better control with it than with insulin mixtures, globin insulin and other modified insulins.

The majority of patients needing 40 units or less of insulin daily can be well regulated with a single daily dose of standard PZ insulin. Of a series of 385 diabetic patients studied during a six year period, 233 (58 per cent) were well controlled with standard PZ insulin. The remaining 152 needed over 40 units daily and could not be regulated with standard PZ insulin, but 90 per cent of this group of severe diabetic patients were well controlled with MPZ insulin. Strength, energy and nutrition are better than with any other treatment program, and the incidence of complications has been reduced. Individual case studies of patients needing as much as 100 units daily illustrate the superiority of MPZ insulin, a more nearly normal 24 hour blood glucose curve being established with an undistorted diet and only one daily injection being required.

41. Effect of Iodine Intake on Thyroid Iodine Distribution and Thyroid Weight of Rats Treated with Thiouracil and Other Goitrogens.

D. A. McGinty and E. A. Sharp. Research Laboratories, Parke, Davis & Company, Detroit.

Absorption of iodine (Rawson and collaborators, 1944) and formation of diiodotyrosine and thyroxin (Franklin and associates, 1944) by the thyroid of the rat is inhibited during thiouracil treatment provided iodine intake is at or near physiological requirements. If, under the same conditions, iodide is added to the drinking water in increasing concentrations, iodine accumulates in the thyroid gland in amounts proportional to increased intakes. Fractionation of thyroids from such animals by means of $Zn(SO_4)_2$ and NaOH indicates, however, that practically all of the iodine exists in non-protein-bound form presumably as inorganic iodide. Since the iodine concentration in other organs under the same experimental conditions was much smaller, Salters suggestion (1945) that a loose combination of iodide and thyroid protein exists, is given support. Equivalent amounts of iodine as iodate or as diiodotyrosine also occur in $Zn(OH)_2$ supernatants if given with thiouracil in the diet. In control experiments without thiouracil, added iodine caused an increase only in the protein-bound fraction of thyroid. The goitrogenic effect of thiouracil as well as that of certain other antithyroid substances studied, persists during high iodine intakes although some decrease in degree of thyroid hypertrophy was noted.

42. The Reversible Inactivation of Thyrotropic Hormone. Its Inactivation by Thyroid Tissue and Reactivation by Thiouracil and Other Goitrogenic Agents.

Rulon W. Rawson, Alexander Albert, Janet W. McArthur, Priscilla Merrill, Beatrice Lennon, and Charlotte Riddell. From the Thyroid Clinic of the Massachusetts General Hospital, Boston.

Explanted slices of normal rabbit thyroids were bathed in Tyrode's solution containing thyrotropic hormone at a temperature of 37°C. After a forty-eight hour period of incubation, the medium was removed and assayed. It was observed that exposure of 10 Junkmann Schoeller units of thyrotropic hormone to slices of one rabbit thyroid resulted in a complete loss of thyroid stimulating effect.

The inactivated hormone was treated with thiouracil and other goitrogenic drugs and assayed. After mixing or after incubation with these agents, the inactivated hormone was observed to recover in varying degrees its thyroid stimu-

lating action. These agents in the amounts used to reactivate the inactive hormone when administered alone had no measurable effect on thyroids of the test animals.

Active hormone after incubation with these goitrogens was found to have a greater thyroid stimulating action than untreated active hormone. The same augmentation was observed after the goiter producing agents had been removed by dialysis.

The theory is advanced that the action of certain goitrogens is at least twofold: that they interfere with thyroid hormone production and that in addition they augment the action of thyrotropic hormone.

43. Sensitivity of the Reproductive System of Hypophysectomized Forty-day-old Male Rats to Testosterone Propionate.

Miriam E. Simpson and Herbert M. Evans. Institute of Experimental Biology, University of California.

In a previous paper (Simpson, M. E., C. H. Li and H. M. Evans: Endocrinology *35:* 96, 1944) it was reported that the testicular tubules of 40-day-old male rats were maintained and even caused to continue development during the 15 days following hypophysectomy when injected with extremely small doses of ICSH. Tubular stimulation, in fact, occurred at doses too low to prevent the accessory organs of reproduction from regressing to the hypophysectomized condition. As it has been assumed that ICSH acts indirectly through the male sex hormone produced, similarly prepared test animals were injected with varying doses of testosterone propionate to compare androgenic with gametogenic effects. This substance (in contrast with ICSH) proved equally good as a stimulant of testicular tubules and accessories. These findings do not sustain the explanation cited for the mode of action of ICSH on the testicular tubules.

44. The Syndrome of Congenitally Absent Ovaries, with Infantilism High Urinary Gonadotropins and Short Stature, with Other Congenital Abnormalities, such as Short Webbed Neck, Cubitus Valgus, Coarctation of the Aorta, etc., and Tabular Presentation of Twenty-One Previously Unpublished Cases.

H. Lisser, L. E. Curtis, Roberto F. Escamilla, and Minnie B. Goldberg. From the Division of Medicine, University of California Medical School, San Francisco, California.

About 50 cases of this syndrome have been published. We are adding 21 cases. The absence of ovaries has been demonstrated in 20 previously reported cases and in 4 of our patients, one of whom was only 13 years old. Urinary gonadotropins tested in 10 of our cases were abnormally high. Two of the remaining 11, however, were verified pathologically. The remaining 9 patients are characteristic clinically. Photographs are available for all but 2 of our 21 cases.

The absence of ovaries is considered responsible for the amenorrhea, absent mammary development, sparse sexual hair and high urinary gonadotropins—but not for the shortness. The latter may be the result of a germinal defect, like the ovarian agenesis and other congenital abnormalities. The shortness would be difficult to explain on a hormonal basis. For contrast, a photograph of a tall female, age 33, who was castrated at the age of 5, will be shown.

Early diagnosis is stressed so that measures to promote growth can be instituted, especially because the "bone-age" is usually only slightly retarded.

Estrogens are highly beneficial in causing breast development, growth of sexual hair, and, if given in cyclic fashion, regular withdrawal bleeding, with consequent profound improvement in morale.

45. Adrenal Ascorbic Acid Response to Adrenocorticotropic Hormone in Intact and Hypophysectomixed Rats.

Paul L. Munson and F. C. Koch. Biochemical Research Division, Armour and Company, Chicago.

Single subcutaneous injections of purified adrenocorticotropic hormone (ACTH) into normal male rats decreased adrenal ascorbic acid concentrations one hour later as follows:

ACTH administered	No. of rats	Adrenal ascorbic acid: decrease from controls
gammas		per cent
2.5–5	36	3
10	29	8
20–25	40	18
40–50	34	20
80	30	22
100	11	42
160–400	32	39

Thirteen successive daily injections of purified ACTH into hypophysectomized male rats raised the ascorbic acid content of the adrenals as follows:

Daily dosage ACTH	No. of rats	Adrenal ascorbic acid
		gammas per rat
Saline only	63	27.4
40 gammas	67	38.2
200 gammas	68	43.7
0.8–1 mg.	28	52.1
Unoperated	13	85.0

When ACTH was administered at a level sufficiently high to increase the adrenal weight above that of unoperated controls the adrenal ascorbic acid of the treated group was still significantly less than that of the controls. Prolongation of the interval between last injection and autopsy from 24 to 48 hours did not change the values significantly.

Although we have demonstrated the existence of a quantitative relationship between ACTH dosage and adrenal ascorbic acid response both in intact and hypophysectomized rats, animal variation is high and the slope of the curve relating log-dosage and response is low. It is concluded that neither procedure will lead to an improvement over bioassay methods previously described. On the other hand, the recently introduced method of Sayers and Sayers (Fed. Proc. *5*, 200 (1946)), using a single intravenous injection of ACTH in one-day hypophysectomized rats, has worked well in our hands, and we believe it shows promise.

46. The Use of Desoxycorticosterone Acetate in a Beeswax Mixture for the Treatment of Addison's Disease.

Charles F. Code, Edward H. Rynearson, and Marschelle H. Power. Section on Clinical Physiology, Division of Medicine, and Section on Clinical Biochemistry, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Beeswax mixtures have been used for the administration of histamine, heparin, penicillin and other substances in cases in which a delay in absorption is advantageous. In the treatment of Addison's disease, desoxycorticosterone acetate has been administered both in oil and as pellets. We have studied its injection in a beeswax mixture in fourteen cases. Three of the patients have died and their

deaths will be discussed. Of the remaining eleven patients, two have had only one or two injections, six have had five to eight injections and three have had ten to twenty injections. The three patients who have had more than ten injections have now been treated for several years by this method. Their experience will be reported in detail.

47. Luteotrophic Action of Chorionic Gonadotrophin in the Woman.

W. E. Brown and J. T. Bradbury. Department of Obstetrics and Gynecology, State University of Iowa.

Two women were given chorionic gonadotrophin (Antuitrin-S) in doses of 20,000 I.U. per day. Treatment was started in the late luteal phase of the menstrual cycle and was continued until the onset of bleeding. Endometrial biopsies were obtained once a week, before, during and after treatment as a means of determining the sequence of changes in ovarian activity. The endometrium of these two women had gone through the normal sequence of changes since the last menstrual period and was a typically premenstrual secretory endometrium at the time of the initial injection. Menstruation was delayed 2 weeks beyond its expected date of onset in one patient, and was delayed 3 weeks in the second patient. The endometrial biopsies obtained during this interval of treatment were definitely secretory and had a marked decidual reaction (deciduoid??). The continued maintenance of this luteal endometrium indicates that the chorionic gonadotrophin has a definite luteotrophic action in the woman. The negative results in our realier report Brown, Bradbury and Metzger (1941) were undoubtedly due to the low doses employed. These studies are being expanded and will be reported in detail.

48. Further Observations on the Absorption of Subcutaneously Implanted Pellets of Hormonally Active Steroids.

Thomas H. McGavack and Herman Reinstein. N. Y. Medical College, Metropolitan Hospital Research Unit.

The rate of absorption of 75 mg. pellets of desoxycorticosterone acetate of constant surface area and hardness has been determined in 15 dogs and in 12 human beings. Similar studies have been made with 75 mg. pellets of testosterone and with 15 mg. pellets of estradiol benzoate in human beings. Experiences with pellets of testosterone implanted within the testicle of 2 human beings will also be detailed.

The over-all time for the absorption of the pellets of desoxycorticosterone acetate was between 10 and 12 months with an average daily absorption per pellet of 0.24 mg. The average period over which patients experienced symptoms was approximately 9 months.

Pellets of testosterone propionate were absorbed at the rate of approximately 0.3 mg. per pellet per day in both the dog and the human being, with an over-all absorption time of 8 to 9 months. Periods of symptomatic relief varied from 2 to 8 months.

Pellets of estradiol benzoate were absorbed at an average rate of about 0.05 mg. per pellet per day with a calculated over-all absorption time of approximately one year. Periods of subjective relief varied from 3 to 9 months.

About the area of testicular implant in one human being there was an active regeneration of testicular tissue. In the other no trace of seminiferous tubules could be found at autopsy some 3 months after a third implantation of 225 mg. of testosterone propionate.

It has been shown that 1.0 mg. desoxycorticosterone acetate absorbed daily from an implanted pellet is the equivalent in activity of approximately 5 mg of the material in a sesame oil medium administered daily by subcutaneous injection. Approximately the same ratio holds for the implantations of testosterone propionate. Sufficient data have not been accumulated to determine the conversion equivalents of implanted pellets of estradiol benzoate in terms of periodic injections

in an oil medium, but it has been possible to produce full estrogenic responses in the vaginal smear from a single 15 mg. pellet.

The number of pellets implanted has no relation to the rate of absorption of the individual pellet. Towards the end of the absorption period there is a decrease in the rate of assimilation, but this is not appreciable for the first five months after implantation. However, it seems unlikely that the decreased absorption is the only factor involved in the return of symptoms and signs of hormonal insufficiency prior to the end of the absorption period.

49. Absence of Renotropic Action of Pure Adrenocorticotropic Hormone (ACTH).

Miriam E. Simpson, Choh Hao Li, and Herbert M. Evans. Institute of Experimental Biology, University of California.

Reiss has recently reported (Reiss, M.: *Nature (London) 154:* 737, 1944) increased kidney weights in young rats acutely treated with a purified pituitary adrenocorticotropic preparation. The conditions of his experiment have been duplicated as closely as possible in an effort to test whether electrophoretically homogeneous ACTH would have the same effect. It was not possible to show changes in renal histology or significant increases in the kidney weights.

50. The Rate of Metabolism of Steroid Hormones by the Livers of Different Species.

L. T. Samuels and C. J. McCauley. Department of Biological Chemistry, University of Utah, Salt Lake City.

The rate of destruction of testosterone and esterone and estrogens has been studied after incubation with liver mince, using chemical methods of estimation. Liver tissue of the human, rat, mouse, rabbit and dog have been used. The rate of destruction is lowest in the human liver and the most rapid with rat and mouse livers. There appears to be no correlation between the rates of destruction of estrone and of testosterone. The bearing of these results on the reactions in different species will be discussed.

51. The Comparative Effect of Various Goitrogenic Agents on the Collection of Radioactive Iodine by the Thyroid in Rats and Chicks.

Rulon W. Rawson, D. A. McGinty and Wendell Peacock. From the Thyroid Clinic of the Massachusetts General Hospital, Boston; the Research Laboratories of Parke Davis & Company, Detroit; and the Nuclear Physics Laboratories, Massachusetts Institute of Technology, Cambridge.

The collection of radioactive iodine by the Thyroids of chicks and rats treated acutely and chronically with various goitrogenic compounds has been compared.

All of the agents studied were alike in preventing the collection of radioactive iodine administered one to six hours after a single injection of the various drugs. However, when administered chronically these agents differed radically in their effects. Thiouracil and related compounds blocked the collection of iodine in both rats and chicks. Amino mercaptothiadiazole and phenylaminomethyl mercaptothiazoline blocked the collection of iodine in rats, but caused an increase in the collection of iodine in chicks. Potassium thiocyanate increased the collection of iodine in rats.

It is concluded that various goitrogenic agents produce their effects on the thyroid through different mechanisms and that the effects produced with such agents vary with the species.

52. Turner's Syndrome: A Case Report.

William M. Moffat. Santa Barbara.

Since the syndrome of infantilism, congenital webbed neck and cubitus valgus in females was first described by Henry H. Turner in 1938 based upon a collection of 10 cases, only 6 cases presenting the entire triad have been published.

This report describes the case of a 21 year old girl of above average intelligence. She is 62¾ inches in height. She has a web of skin extending from behind each ear to the shoulder. The posterior surfaces of these webs are covered with thick hair. She has cubitus valgus. The external genitalia are undeveloped; no uterus is palpable and the cervix is about 5 mm. in diameter. No glandular tissue can be felt in her breasts which are rudimentary with small areolae and no nipples. Her hips are flat like a boy's. Her skin displays many pigmented moles. Vaginal smears show that the mucosal cells are completely lacking in glycogen. X-ray studies show an epiphyseal development of 16 years of age. The relationship of Turner's Syndrome to other reported syndromes presenting some of the same features is discussed.

53. Influence of Metabolism on Enuresis.

Robert M. Oslund. Ross-Loos Medical Group, Los Angeles.

Nocturnal enuresis appears to be a functional disturbance resulting from or accompanying low metabolism.

Patients ranging in age from 3¼ to 19 years, studied for abnormal physiological bone age, were not indicative. Basal metabolic readings were usually only slightly below normal; however, thyroid given to these patients produced cessation of nocturnal enuresis within two to three weeks; similar results although not as rapid, may be obtained from pituitary material.

As a great many hypothyroid patients do not experience nocturnal enuresis, the results are therefore interpreted as purely metabolic and not a thyroid deficiency.

54. Quantitative Inhibition of Oestrogen Effect on Chick Oviduct by Progesterone.

Roy Hertz. National Institute of Health, Bethesda, Md.

Inhibition of estrogen-induced weight increase in the chick oviduct by simultaneously administered progesterone is proportional to the progesterone dosage up to a maximum, beyond which even a six-fold increment in progesterone level effects no further inhibition. In the presence of a maximally effective progesterone dosage a sixteen-fold increase in estrogen dosage does not overcome the progesterone inhibition. Preliminary administration of maximally effective doses of progesterone for two days prior to the initiation of estrogen treatment does not enhance the degree of inhibition obtained.

Homogenates of the oviduct from chicks treated with an inhibitory combination of estrogen and progesterone are without effect upon the estrogen response in the chick. Additional studies on the mechanism of the estrogen-progesterone antagonism will be reported.

55. The Effect of Subcutaneous Administration and Topical Application of Steroid Preparations to the Pigmentation Area of the Hamster.

Herbert S. Kupperman. Department of Endocrinology, University of Georgia School of Medicine, Augusta.

In the costo-vertebral area of the golden hamster an area of excessive melanin deposition has been observed in the adult male hamster. This area is about 0.5 cm. in diameter, also associated with an increased amount of coarseness of the pellage. Pigmentation is absent or diminished in immature and adult females, immature and castrated males, and is of decreased intensity in senile males. Pigmentation and coarseness of the tuft of hair may be induced by topical application or subcutaneous administration of androgenic preparations. Estrogen, progesterone and desoxycorticosterone have a suppressing effect on the pigmented area. Simultaneous administration of androgen and estrogen to castrated males produces essentially the same effect as androgens alone, thus failing to demonstrate antagonism between folliculoid and android hormones. The relative effec-

tiveness of the steroid preparation in producing pigmentation in the castrated male or female is closely correlated with the android activity of the preparation. The topical application of alcoholic solution of male hormone as a means of assay for android activity is discussed.

56. The Influence of the Adrenal Cortex on the Metabolism of the Eviscerated Rat.

Sidney Roberts. From the Worcester Foundation for Experimental Biology, Shrewsbury.

Adrenalectomy following evisceration in the fasted rat produced a 50 percent reduction in survival time and an increased rate of blood sugar disappearance. Upjohn cortical extract in oil had a small moderating effect. Other extracts tested were still less active. Since estrogens have been found to be inactive under similar circumstances (Szego & Roberts, Endocrinology *36:* 104, 1945), the viscera may be essential for the complete activity of steroid hormones in general.

Adrenalectomy in the eviscerated rat did not influence:

(1) rate of rise of blood amino acids,

(2) rate of fall of blood volume,

(3) simultaneous renal arterio-venous (A-V) differences for sugar, amino acids, and hemoglobin.

Simultaneous analyses of tail and aortal blood samples for sugar revealed that adrenalectomy uniformly produced an increased peripheral A-V difference. This might be due to more rapid sugar utilization. Other considerations, however, indicate that the primary effect of adrenalectomy in the eviscerated rat is hemodynamic, and that the increased peripheral A-V difference reflects an increased circulation time. The circulatory changes include a reduction in renal blood flow, which suggests that the actual rate of gluconeogenesis in the kidney is reduced by adrenalectomy even though renal A-V differences for sugar are unchanged.

57. The Nature of Benadryl Activity: Glucose Tolerance Curves.

Herman Reinstein and T. H. McGavack.

N. Y. Medical College, Metropolitan Hospital Research Unit.

Intravenous glucose tolerance curves have been performed by standard techniques on 17 subjects ranging in age from 21–71 years. Each patient was maintained on a diet of 2300 calories, containing 200 gm. of carbohydrate, 95 gm. of protein, and 125 gm. of fat, for 3 days before the performance of the first glucose tolerance test and throughout the entire period of study except on the days during which tests were taken. Three tests were done on each subject: one without Benadryl (Curve I), one beginning one-half hour following the ingestion of 400 mgm. of Benadryl orally (Curve II), and the third, one-half hour following the administration of 30 mgm. of Benadryl intravenously (Curve III). The results of each of the three tests have been averaged for the 14 patients. In each instant a composite curve was drawn; the first and second of these composite curves closely approximate each other. However, it was noted that in younger individuals the area of the curve obtained following Benadryl orally was significantly larger than that of the control, whereas in older individuals, above 50, the reverse was true. Following the use of Benadryl intravenously (Curve III) there was a significant increase in sugar tolerance in all subjects irrespective of age, as shown by a decrease in the areas of the individual and composite curves respectively. In order to rule out any normal variation in glucose tolerance when determined at three day intervals, three subjects were given the same regime as the 14 test subjects mentioned above except that no medication was used in conjunction with any of the three tests obtained per subject. It is significant that in each of these three subjects curves of smallest area were obtained in the first test with a moderate but sigfinicant increase in the area with each of the two succeeding examinations.

These findings will be discussed in relation to the nature of the action of Benadryl and its antihistamine effects.

58. Nature and Distribution of the Skeletal Abnormalities in Cushing's Syndrome.

Fuller Albright, Anne P. Forbes, and Edward C. Reifenstein, Jr. Dept. of Medicine, Harvard Medical School and Massachusetts General Hospital, Boston.

It is pointed out that the demineralization of the bone in Cushing's Syndrome is the result of osteoporosis. By "osteoporosis" is meant that bone condition where the primary abnormality is a failure of the osteoblasts to lay down matrix.

The osteoporosis in Cushing's Syndrome has a marked predilection for the spine and pelvis. A case of Cushing's Syndrome in a girl of thirteen is presented. The x-rays of the bone before and after the condition was alleviated are most instructive.

The effect of potassium on the calcium balance of two cases of Cushing's Syndrome are presented.

59. Management of Threatened and Habitual Abortion by Large Doses of Oral Estrogen.

A. R. Abarbanel. Department of Obstetrics and Gynecology College of Medical Evangelists, Los Angeles.

Fifty cases of threatened and habitual abortion were treated by means of oral estrogen (diethylstilbestrol) in doses ranging from 15 to 500 mg. daily. The homostatic effect was evident in 4 to 24 hours.

A large percentage of fetuses were salvaged. It is felt that the effect of estrogen upon the endometrial vascular bed, particularly at the site of placentation, is responsible for the favorable clinical results.

60. Clinical Syndromes of Aberrations in Corpus Luteum Function.

Virgil O. Parret and A. R. Abarbanel. From Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles.

The following syndromes which are felt to stem from aberrations of corpus luteum function are briefly presented and correlated with the pathological findings:

Hemorrhage into corpus luteum (simulating an acute surgical abdomen).
Persistent corpus luteum simulating ectopic pregnancy.
Lutein cysts resembling chocolate cysts of endometriosis.
Persistent corpus luteum causing prolonged amenorrhea of several years.
Luteinized thecoma and also luteoma causing masculinization syndrome.

61. Problem of "Closed Fallopian Tubes" Hormonal Differentiation of Tubes Closed by Spasm from Those Closed by Organic Pathology by Means of Methyl Testosterone.

A. R. Abarbanel. Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles.

Twelve patients, who were diagnosed as having "closed tubes" and advised that they were now sterile, were given oral methyl testosterone, 5 mg. sublingually per day for one to three months. In each case the tubes were then found to be patent. In four cases pregnancy ensued during therapy.

The modus operandi is discussed.

62. The Use of Sodium Para-Aminobenzoate Parenterally in the Treatment of Hyperthyroidism.

Louis Berman, N. Y. City.

In a preliminary communication to the Sodiety for Experimental Biology and Medicine in May, 1945, a report was presented of beneficial therapeutic effects obtained in six ambulatory cases of hyperthyroidism by the parenteral administra-

tion of one gram or larger doses of the sodium salt of para-aminobenzoic acid six times weekly over a period of months with no unfavorable by-effects such as have been reported by clinicians employing thiourea and thiouracil. Eight more cases of hyperthyroidism so treated will be reported.

The treatment of the first case was begun in 1943, a patient with hyperthyroidism complicated with vitiligo to whom the sodium salt was administered as an attempt to influence the skin pigmentation disturbance. The oral use of para-aminobenzoic acid by itself and as a supplement to the parenteral administration of its sodium salt will also be discussed. Observations of the effects on pulse, blood pressure, weight, basal metabolism, blood cholesterol and the clinical symptoms and course of the disease will be presented. The details of the eight other cases will be given in addition to further observations on the six originally treated and followed up.

63. Hypogonadotrophic Enuchoidism—Its Clinical and Laboratory Delineation from Other Forms of Hypogonadism with a Discourse on Treatment.

Carl G. Heller and Warren O. Nelson. Departments of Physiology and Medicine, University of Oregon Medical School, Portland, and Department of Anatomy, University of Iowa, Iowa City.

Gonadotrophic hormone assays and testicular biopsies were found to be the most reliable laboratory criteria for establishing the diagnosis of hypogonadotrophic eunuchoidism. After laboratory classification it was possible to delineate this group from other forms of eunuchoidism by clinical means and by use of a therapeutic test.

Successful correction occurred following administration of chorionic gonadotrophin and a purified follicle stimulating hormone (prepared by McShan). Response was judged by clinical improvement and laboratory and serial biopsy changes.

64. Determination of Corticosteroids in Urine.

B. E. Lowenstein, A. C. Corcoran, and Irvine H. Page. From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio.

The present method is based on periodate oxidation (Fieser, Fields and Lieberman) of the primary alcohol group at C_{21} which yields one mol of formaldehyde per mol of corticosteroid oxidized. Formaldehyde is then determined by the method of MacFadyan.

Procedure: To a 3 cc. aqueous aliquot of a urinary extract (Venning, Hoffman and Browne) is added 0.1 cc. of 0.05 M HIO_4 in 0.3 N H_2SO_4. Oxidation proceeds at about 25°C for 20 minutes when it is arrested by addition of 0.1 cc. of 6 per cent $SnCl_2$. Five cc. of 0.2 per cent chromotropic acid (Eastman) in 15 M H_2SO_4 are added and the mixture heated for 30 minutes at 100°C. The cooled solution is made up to 12.5 cc. by addition of 9 M H_2SO_4 and color density read in the Coleman Model 6 spectrophotometer.

The method gives good recoveries from urine samples containing added cortical extract. The average excretion of corticosteroids in normal males is .5–.8 mg. per 24 hours, expressed as dehydrocorticosterone. In a case of Addison's disease, the value was 0.15 mg. and in one of Cushing's syndrome, 21 mg. per 24 hours.

The advantages of the method lie in simplicity, lack of urinary "blank" and in that the estimates include both active and partially reduced corticosteroids.

65. The Effect of Hypophysectomy on the Time of Lay of the Hen's Egg.

Irving Rothchild (Introduced by Richard M. Fraps). U. S. Department of Agriculture, Agricultural Research Center, Beltsville, Maryland.

Delays in lay occurred in Rhode Island Reds and White Leghorns, after either complete or partial hypophysectomy performed either on the day of ovulation or

on the day of expected lay; no delays however, occurred after sham hypophysectomy. The distribution of results according to breed, completeness of operation, and time of operation was as follows:

	Rhode Island Reds	White Leghorns
Complete hypophysectomy on day of ovulation	delays in 12 out of 25 birds	delays in 9 out of 27 birds
Complete hypophysectomy on day of expected lay	delays in 4 out of 9 birds	delays in 1 out of 10 birds
Partial hypophysectomy on day of ovulation	delays in 8 out of 20 birds	delays in 1 out of 7 birds
Partial hypophysectomy on day of expected lay	delays in 2 out of 5 birds	delays in 2 out of 3 birds
Sham hypophysectomy on day of ovulation	no delays in 16 birds	no delays in 15 birds
Sham hypophysectomy on day of expected lay	no delays in 4 birds	

These data indicate that removal of or injury to the AP results in partial interference with the determination of the time of normal lay. The nature of AP participation in the process of lay is under further investigation.

66. Parathyropituitary Syndrome in Pituitary Basophilism.
Robert M. Perlman. San Francisco.

Pathologic bone changes are an almost invariable accompaniment of pituitary basophilism. In earlier papers discussion centered around the probability that such changes are the result of a "primary" pituitary malady which secondarily affects the parathyroid glands, upsetting the calcium metabolism, and thereby leading to disturbances in bone structure. It was shown that an intricate parathyropituitary relationship also occurred in pituitary eosinophilism, resulting in a definite symptom-complex which was later described as the "Parathyropituitary Syndrome in Pituitary Eosinophilism." Altogether special mechanisms must enter into play when basophilic hyperpituitarism occurs simultaneously with hyperparathyroidism. Such mechanisms must be investigated separately and interpreted clearly; hence, *raison d'être* for this present paper.

Substantial evidence is presented in the body of this paper to indicate that the basophilic version of the parathyropituitary syndrome may find expression in either of two extreme or a multitude of intermediate forms: 1) Cushing's syndrome in its banal form, with relatively mild skeletal destruction ("osteoporosis and osteomalacia" leading to scoliosis, kyphoscoliosis, lordosis, etc.). 2) Cushing's syndrome complicated by an accentuated osteoclastic skeletal resorption and fibrous neoformation of osseous tissues. These processes can become intense enough to render the full-fleged, typical, clinical and anatomical picture of von Recklinghausen's bone disease complete with spontaneous fractures and adenomatous parathyroid hyperplasis. The Syndrome may express itself in varying degrees, and may be complicated further by the intervention of renal pathology.

67. Diurnal Variations in the Levels of Blood Glucose of Normal Human Beings Subjected to Various Types of Caloric Restriction.
David Schwimmer and Thomas H. McGavack. N. Y. Medical College, Metropolitan Hospital Research Unit.

Fifty-seven subjects have been divided into groups and given varying amounts of carbohydrate, protein, and fat with total caloric equivalents ranging from 900–1800 calories. The protein of the diet varied from 0–10% by weight. The subjects were fed at 9 A.M., 1 P.M., 5 P.M., and 9 P.M., with divisions of $\frac{1}{6}$, $\frac{1}{3}$, $\frac{1}{3}$ and $\frac{1}{6}$, respectively. The widest fluctuations in blood glucose were observed in subjects who received diets containing only carbohydrate and fat. This was true irrespective of what caloric equivalent was used. Many of the subjects on these diets showed

hypoglycemic symptoms and the signs came on as a rule between one-half and two hours after the 1 P.M. feeding. Simultaneously, levels for blood sugar dropped to levels as low as 50 mgm. per 100 cc. Sources of protein supplements were malted milk, lactalbumin, and dried egg white. The amino-acid methionine was similarly tried in lieu of and with protein. Methionine alone had no significant influence on the blood sugar trend. All forms of protein decreased the range of variation in the levels for the glucose of the blood.

When the above findings were brought into relation with data simultaneously procured regarding urinary volume, solutes, nitrogen balance, electrolyte balance and so forth, it was concluded that protein may serve a very useful purpose as a part of restricted rations.

68. THE ROLE OF SEX HORMONES IN THE ORIGIN AND DEVELOPMENT OF ENDOMETRIAL GLANDS IN THE OPOSSUM.

Carl R. Moore and Elizabeth A. Failor. Hull Zoological Laboratory, The University of Chicago.

In the young opossum uterine development is slow hence is excellent for the determination of sex hormonal influences. Muellerian ducts are formed between postnatal days 3 and 10; oviducts, uterus and lateral vaginal canals are distinguishable by day 30. Uterine growth and differentiation continue progressively and by day 80 the smooth epithelial lining changes to low villi and shallow crypts. Endometrial glands arise as balls of cells from base of crypts about days 85–95 and hollow out into small flask-shaped glands by day 100. Rapid gland formation and elongation characterize postnatal days 100–140.

Bilateral ovariectomy on day 20 fails to delay uterine differentiation up to the time of usual gland formation (days 85–95) but it does prevent gland formation. Ovariectomy from days 80 to 95 causes a cessation of gland development even when glands are in process of development at operation.

Androgens as well as estrogens stimulate endometrial gland formation precociously as early as day 40 but sensitivity of the uterus to respond increases as the time of gland formation is approached.

It becomes clear that 1) endometrial gland formation and development is a function of estrogenic hormones from the ovary; 2) this is not an estrogen specific response but occurs with androgens; 3) the earliest detectable ovarion secretion occurs around days 85–95; 4) early uterine differentiation does not depend upon secretions from the developing ovary.